Supplementary Data

A disease-inspired in vitro model of aortic valve stenosis to investigate the drivers of endothelial-mesenchymal transition

Yasmin Mirzaalikhan^{1,2}, Austin Lai², Manijeh Khanmohammadi^{1,2}, Chanly Chheang²,

Azadeh Mirabedini³, Shadi Houshyar³, Jonathan Noonan^{2,4}, Anna M.D. Watson^{2,4,5}, Nalin

Dayawansa^{2,6,7}, Silvana Marasco^{7,8}, Karlheinz Peter^{2,4,6}, Sara Baratchi ^{1,2,4} *

- 1. School of Health and Biomedical Sciences, RMIT University, Melbourne, Victoria, Australia.
- 2. Baker Heart and Diabetes Institute, Melbourne, Victoria, Australia.
- 3. School of Engineering, RMIT University, Melbourne, Victoria, Australia
- 4. Department of Cardiometabolic Health, University of Melbourne, Parkville, Victoria, Australia.
- Department of Diabetes, School of Translational Medicine, Monash University, Victoria, Australia
- 6. Department of Cardiology, The Alfred Hospital, Melbourne, Victoria, Australia.
- 7. Central Clinical School, Monash University, Melbourne, Victoria, Australia.
- 8. Department of Cardiothoracic Surgery, The Alfred Hospital, Melbourne, Victoria, Australia.

*Corresponding author: A/Prof. Sara Baratchi: sara.baratchi@baker.edu.au

Hydrogels	ColMA	ColMA+2mg/ml	ColMA+10mg/ml
	(6mg/ml)	HAMA	HAMA
Approx. Young's modulus At 100 Hz	13257.96	16815.57	18767.85

Table S1. Hydrogel conditions and their corresponding Young's modulus values.



b	vWF	Static α-SMA	Overlay
ColMA			
ColMA-HA2	1		
ColMA-HA10	7		
b'	vWF	10% α-SMA	Overlay
ColMA			
ColMA-HA2			
ColMA-HA10			
b"	vWF	20% α-SMA	Overlay
ColMA			
ColMA-HA2			
ColMA-HA10			<u>50µт</u>

Figure S1. Representative microscopy images related to Figure 4. Confocal images of HAoVECs cultured for 24 hours on different hydrogels and subjected to overnight cyclic stretch illustrate the combined effects of HA deposition and mechanical stress on EndMT. Cells were stained for CD31 and MMP9 markers under different stretch levels (a-a") as well as for vWF and aSMA markers (b-b"). Under static conditions, increased GAG concentrations elevated endothelial markers (CD31, vWF) and mesenchymal markers (MMP9, α -SMA), suggesting that GAGs promote EndMT. In contrast, exposure to 10% and 20% cyclic stretch decreased the expression of these markers compared to static conditions.



Figure S2. Change in stretch intensity or HA concentration does not affect the overall MMP activity. Bar graph showing the overall MMP activity of HAoVECs cultured for 24 hours on different hydrogels and subjected to overnight cyclic stretch. Data were analysed using two-way ANOVA followed by Tukey's multiple comparisons test.