Electronic Supplementary Material (ESI) for Biomaterials Science.

1 Supplementary Information

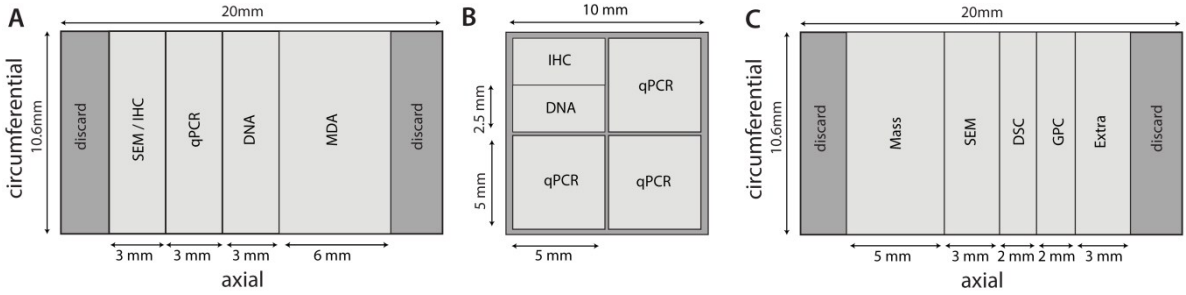

Fig. S1 Experimental cutting schemes. Cutting schemes used for the macrophage seeded samples at day 8 **(A)**, the HVSCs-seeded samples at day 4 and 8 **(B)** and the cell-free samples at day 8 **(C)**, respectively. Abbreviations: scanning electron microscopy (SEM), immunohistochemistry (IHC), quantitative polymerase chain reaction (qPCR), malondialdehyde (MDA), differential scanning calorimetry (DSC), gel permeation chromatography (GPC).

Supplementary Table 1. Primers for gene expression analysis

Primer	Symbol	Accession number	Primer Sequence (‘5-‘3)	MQs	HVSCs
Phenotypic markers					
Cluster of differentiation 68	CD68	NM_001251.3	FW: TTCACCAGCTGTCCACCTC RV: CACTGGGGCAGGAGAAACT	x	
Monocyte chemoattractant protein 1	MCP1 (CCL2)	NM_002982	FW: CAGCCAGATGCAATCAATGCC RV: TGGAATCCTGAACCCACTTCT	x	x
Chemokine (C-C motif) receptor 7	CCR7	NM_001838	FW: AAGCCTGGTTCCTCCCTATC RV: ATGGTCTTGAGCCTCTTGAAATA	x	
Tumor necrosis factor alpha	TNF	NM_000594	FW: GAGGCCAAGCCCTGGTATG RV: CGGGCCGATTGATCTCAGC	x	
Interleukin 6	IL6	NM_000600	FW: ACTCACCTCTTCAGAACGAATTG RV: GTCGAGGATGTACCGAATTTGT		x
Mannose receptor c, type 1	CD206 (MRC-1)	NM_002438	FW: TGGGTTCCTCTCTGGTTTCC RV: CAACATTTCTGAACAATCCTATCCA	x	
Cluster of differentiation 163	CD163	NM_004244	FW:CACTATGAAGAAGCCAAAATTACCT RV: AGAGAGAAGTCCGAATCACAGA	x	
Interleukin 10	IL10	NM_000572	FW: GACTTTAAGGGTTACCTGGGTTG RV: TCACATGCGCCTTGATGTCTG	x	
Transforming growth factor, beta 1	TGFB1	NM_000660	FW: GCAACAATTCCTGGCGATACCTC RV: AGTTCTTCTCCGTGGAGCTGAAG	x	x
α smooth muscle actin	ACTA2	NM_001613.1	FW: CGTGTTGCCCCTGAAGAGCAT RV: ACCGCCTGGATAGCCACATACA		x
Smoothelin	SMTN	NM_134270	FW: CAGCCCAGAACCGAGAGTC RV: AGCAGCCATAGGAGAATCAGAT		x
Calponin	CNN1	NM_001299.5	FW: TTGAGGCCAACGACCTGTTT RV: TTTCCGCTCCTGCTTCTCTG		x
Vimentin	VIM	NM_003380	FW: AAGACCTGCTCAATGTTAAGATC RV: CTGCTCTCCTCGCCTTCC		x
Tissue formation					
Collagen type I	COL1A1	NM_000088	FW: AATCACCTGCGTACAGAACGG RV: TCGTCACAGATCACGTCATCG		x
Collagen type III	COL3A1	NM_000090	FW: ATCTTGGTCAGTCCTATGC RV: TGGAATTTCTGGGTTGGG		x
Lysyl Oxidase	LOX	NM_002317.3	FW: CCTGGCTGTTATGATAC RV: GAGGCATACGCATGATG		x
Elastin	ELN	NM_000501.3	FW: CTGGAATTGGAGGCATCG RV: TCCTGGGACACCAACTAC		x
Fibrillin 1	FBN1	NM_000138.4	FW:TGTTGGTTTGTGAAGATATTG RV: GTGGAGGTGAAGCGGTAG		x
Fibrillin 2	FBN2	NM_001999	FW:ATCCCTGTGAGATGTGTC RV: TTCCTCCTTGGCATATCC		x
Decorin	DCN	NM_133503	FW:TGCAGCTAGCCTGAAAGGAC RV: TTGGCCAGAGAGCCATTGTC		x

Versican	VCAN	NM_004385	FW:GGCACCTGTTATCCTACTGAAA RV: ACACAAGTGGCTCCATTACG		x
Remodeling					
Matrix Metalloproteinase 1	MMP1	NM_001145938.1	FW:CGCACAAATCCCTTCTACCC RV: CTGTCGGCAAATTCGTAAGC		x
Matrix Metalloproteinase 2	MMP2	NM_001127891	FW: ATGACAGCTGCACCACTGAG RV: ATTTGTTGCCCAGGAAAGTG		x
Matrix metalloproteinase 9	MMP9	NM_004994	FW: TGGGGGGCAACTCGGC RV: GGAATGATCTAAGCCCAG	x	
Metallopeptidase inhibitor 1	TIMP1	NM_003254.2	FW: TGACATCCGGTTCGTCTACA RV: TGCAGTTTTCCAGCAATGAG		x
Metallopeptidase inhibitor 2	TIMP2	NM_003255.4	FW:GGAGGAATCGGTGAGGTC RV: AACAGGCAAGAACAATGG		x
ROS and enzyme production					
Nicotinamide adenine dinucleotide phosphate-oxidase 2	NOX2 (CYBB)	NM_000397.3	FW:AACTGGGCTGTGAATGAGGG RV: GCCAGTGCTGACCCAAGAA	x	
Nuclear factor kappa-light-chain-enhancer of activated B cells	NFKB1	NM_001165412	FW:AGACCAAGGAGATGGACCTCA RV: GCATTGGGGGCTTTACTGTC	x	
Lipase A or cholesterol ester hydrolase	LIPA	NM_001288979.1	FW:TCCTGCTGGAACTTCTGTGC RV: ACTGCTTCCCCAGTCAAAGG	x	

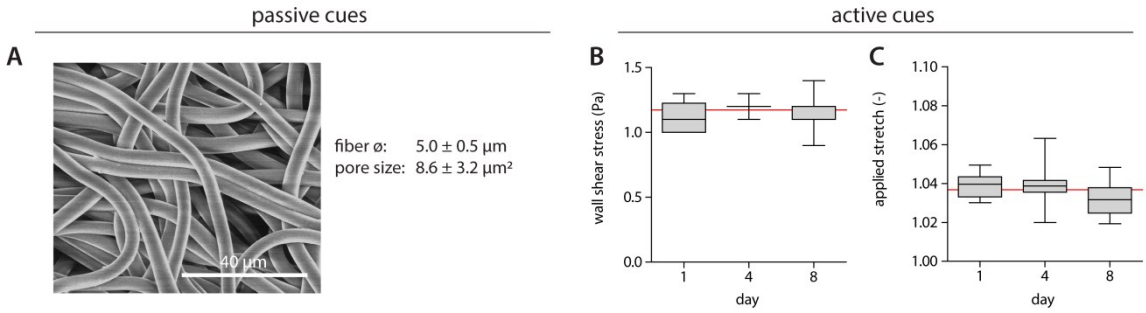

Fig. S2 Characterization of scaffolds and hemodynamic loads. Representative SEM image of the topography plus the calculated average fiber diameter (Ø, µm) and pore size (µm^2) **(A)**; Quantification of the wall shear stress **(B)** and cyclic stretch **(C)** applied during the experiment.

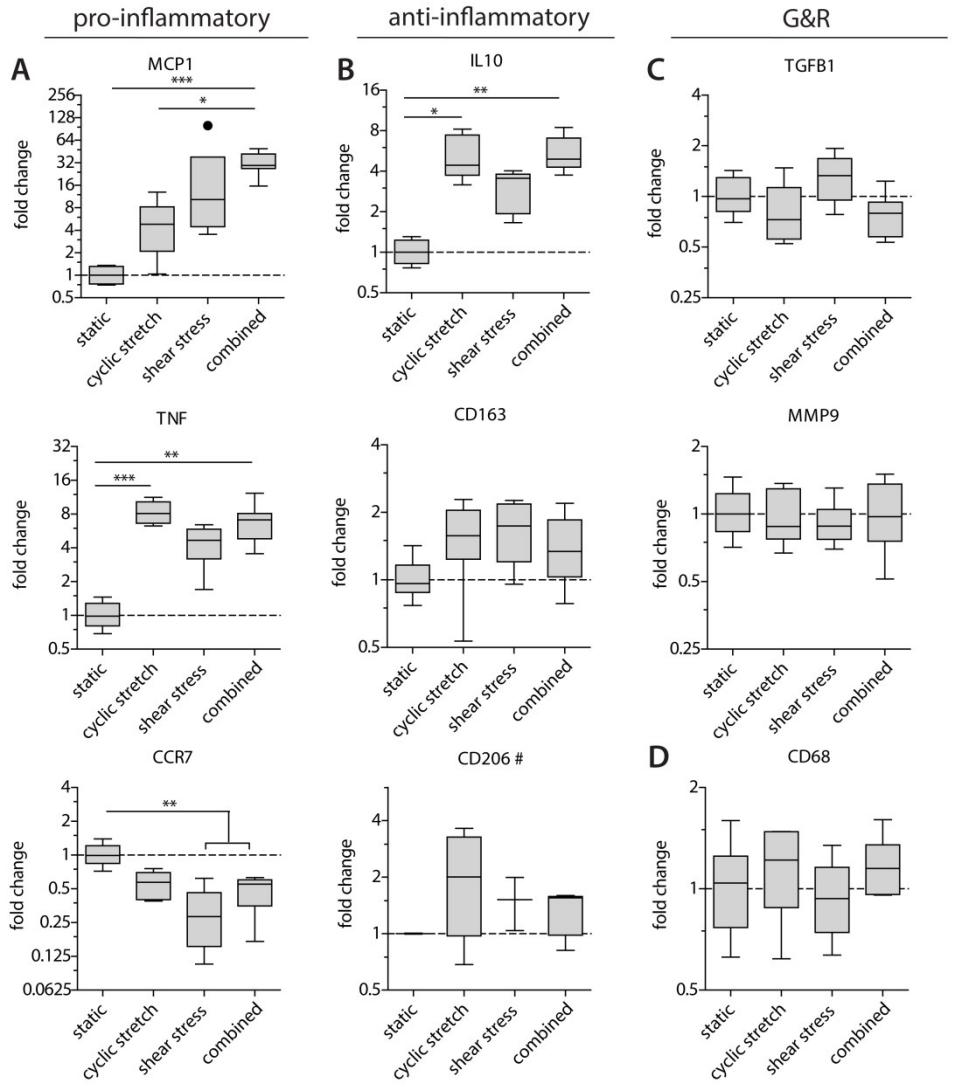

Fig. S3 Gene expression profiles of the statically and dynamically cultured macrophages at day 8. Boxplots representing the fold changes in relative gene expression for the pro- **(A)** and anti-inflammatory markers **(B)**, growth and remodeling (G&R) **(C)** as well as the pan-macrophage marker CD68 **(D)**, when compared to the statically cultured control. The dot represents a statistical outlier. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. $n \geq 5$ / group. # $n \geq 2$ / group.

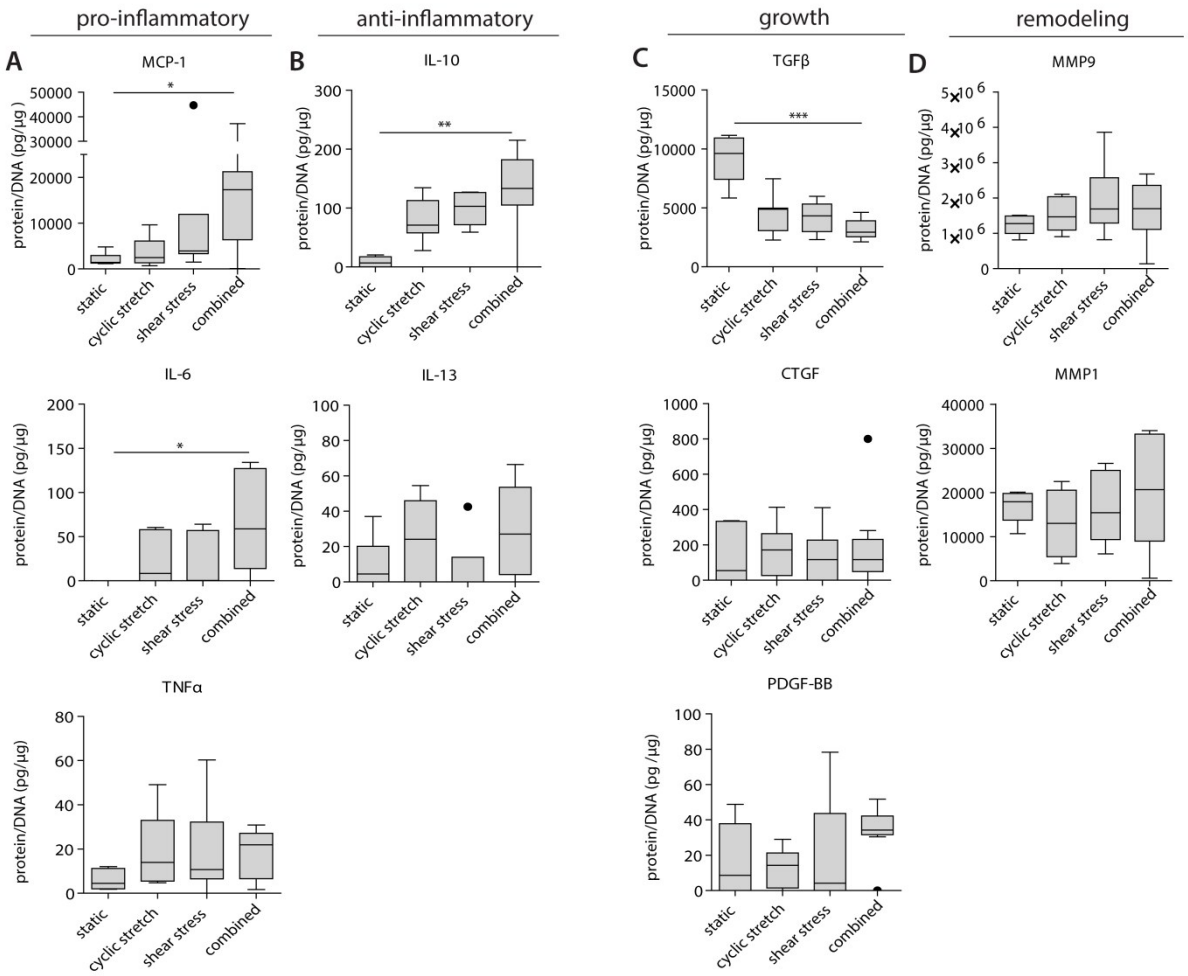

Fig. S4 Protein secretion profiles of the statically and dynamically cultured macrophages at day 8. Boxplots visualizing the secretion levels of a selection of pro- **(A)** and anti-inflammatory proteins **(B)**, as well as proteins related to growth **(C)** and remodeling **(D)** (G&R). The dot represents a statistical outlier. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. $n \geq 5$ / group / time point. See supplementary Fig.S5 for the uncorrected data at day 4 and 8.

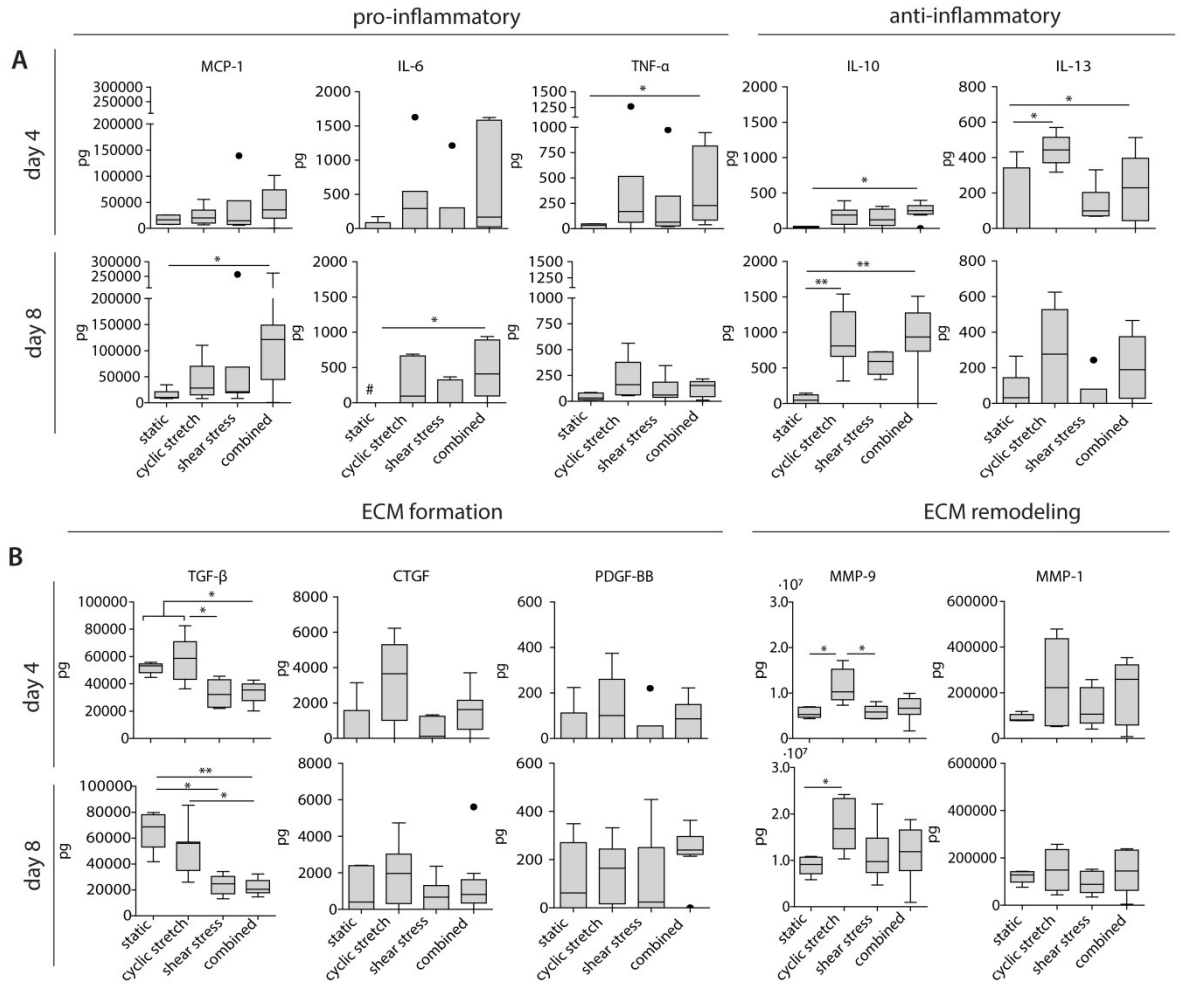

Fig. S5 Cytokine secretion in the medium. Total cytokine secretion levels in pg for the pro- and anti-inflammatory **(A)** and ECM-related cytokines **(B)** at day 4 and 8 of the statically and dynamically cultured macrophages. The dot represents a statistical outlier. * $p < 0.05$; ** $p < 0.01$, $n \geq 5$ / group / time point.

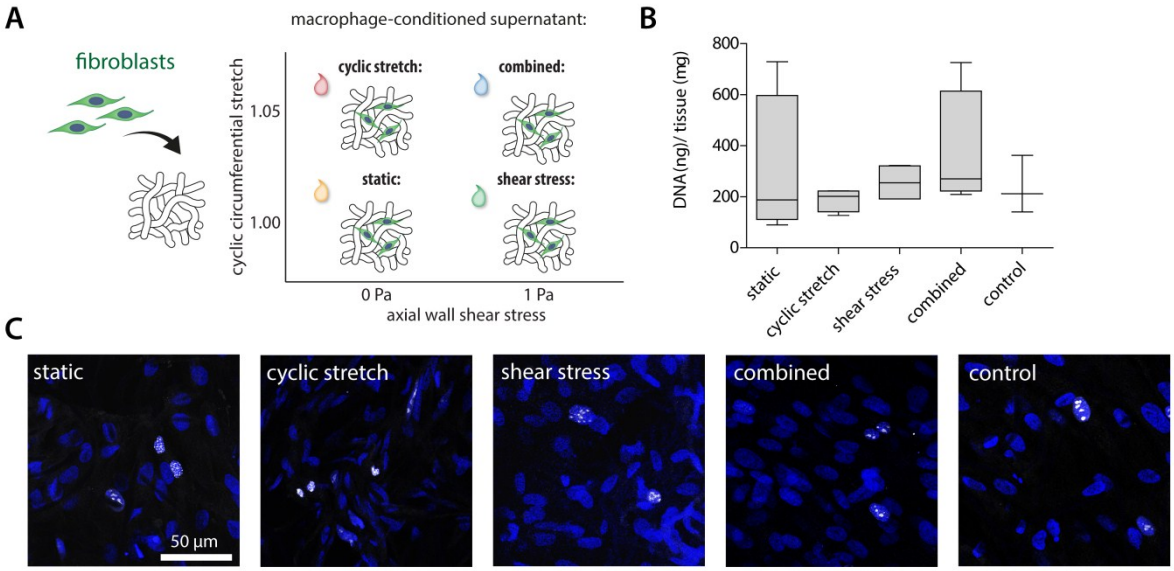

Fig. S6 HVSCs proliferation and cell number after 8 days exposure to macrophage conditioned medium. Representative Ki67 stainings of the HVSCs exposed to conditioned medium or fresh medium (indicated as *control*) (whole-mount staining, overlay of z-stack ± 25 µm, *blue* = DAPI; *white* = Ki67) **(A)**. Quantification of the DNA content normalized to overall construct mass **(B)**. n ≥ 3 / group.

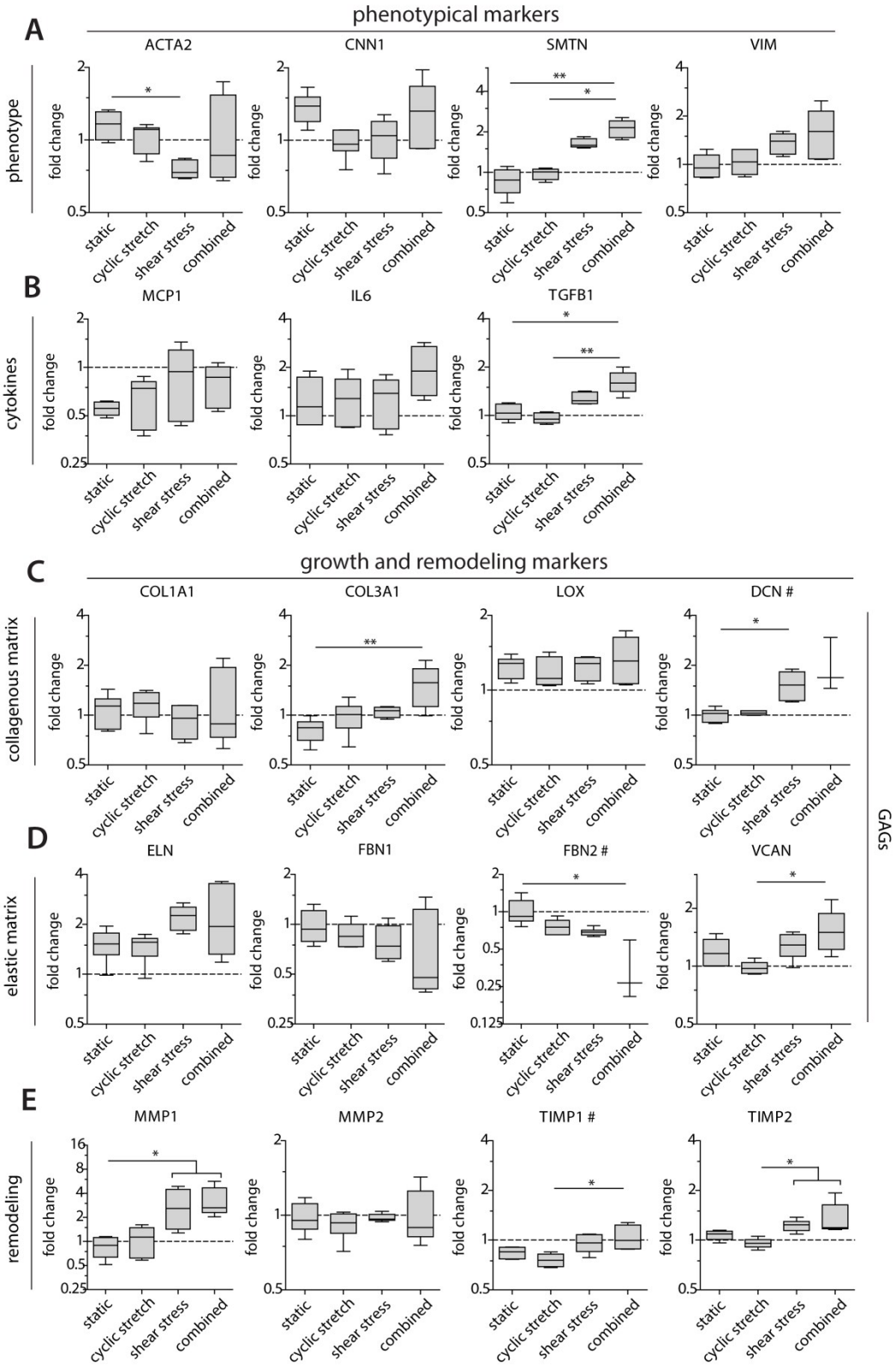

Fig. S7 Gene expression profiles of the HVSCs at day 8. Boxplots visualizing the fold changes in relative expression of the (myo)fibroblast phenotypical markers **(A)**, cytokines **(B)** and markers

related to collagenous **(C)**, GAGs / proteoglycans **(C,D)** and elastic matrix deposition **(D)** or matrix remodeling **(E)** in comparison to the (myo)fibroblast samples that were cultured in fresh medium (control, indicated by the dashed line). * $p < 0.05$; ** $p < 0.01$. $n \geq 5$ / group.