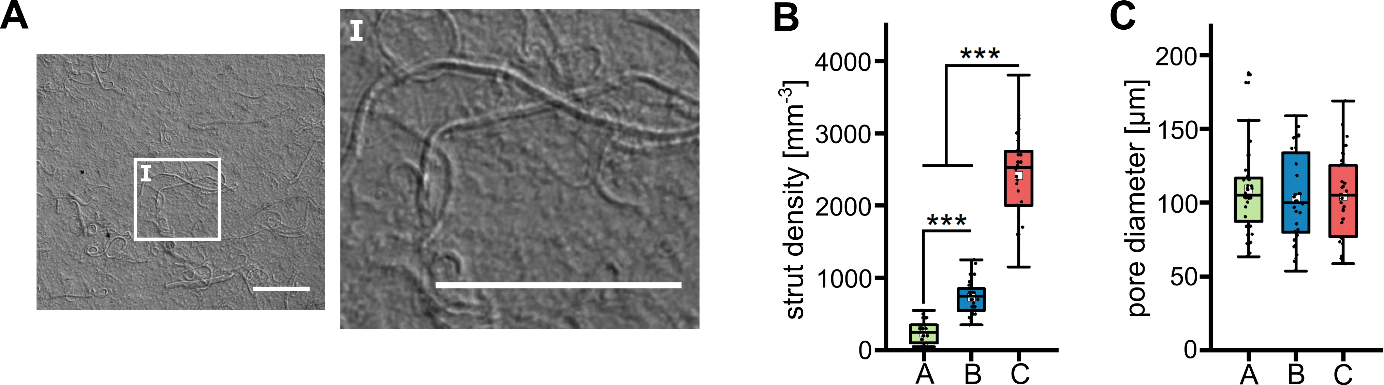
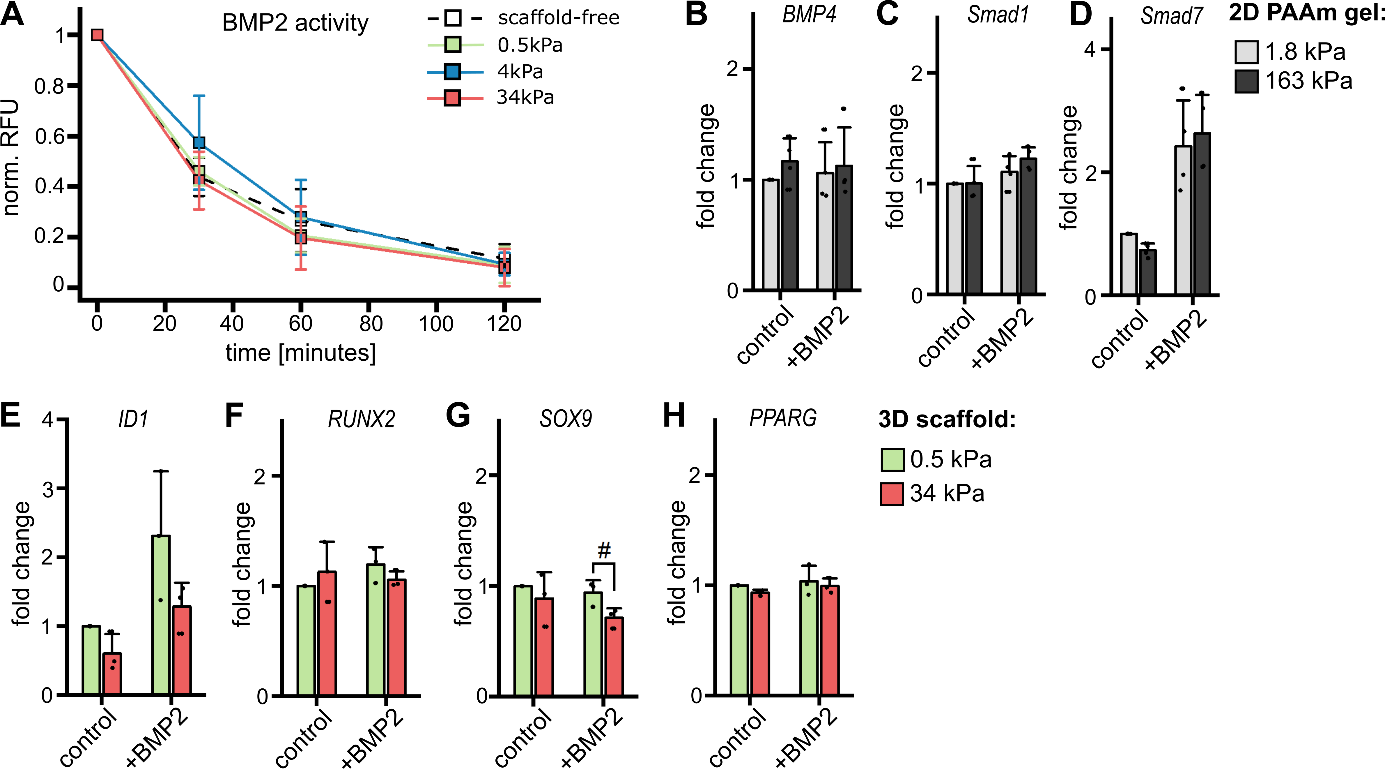
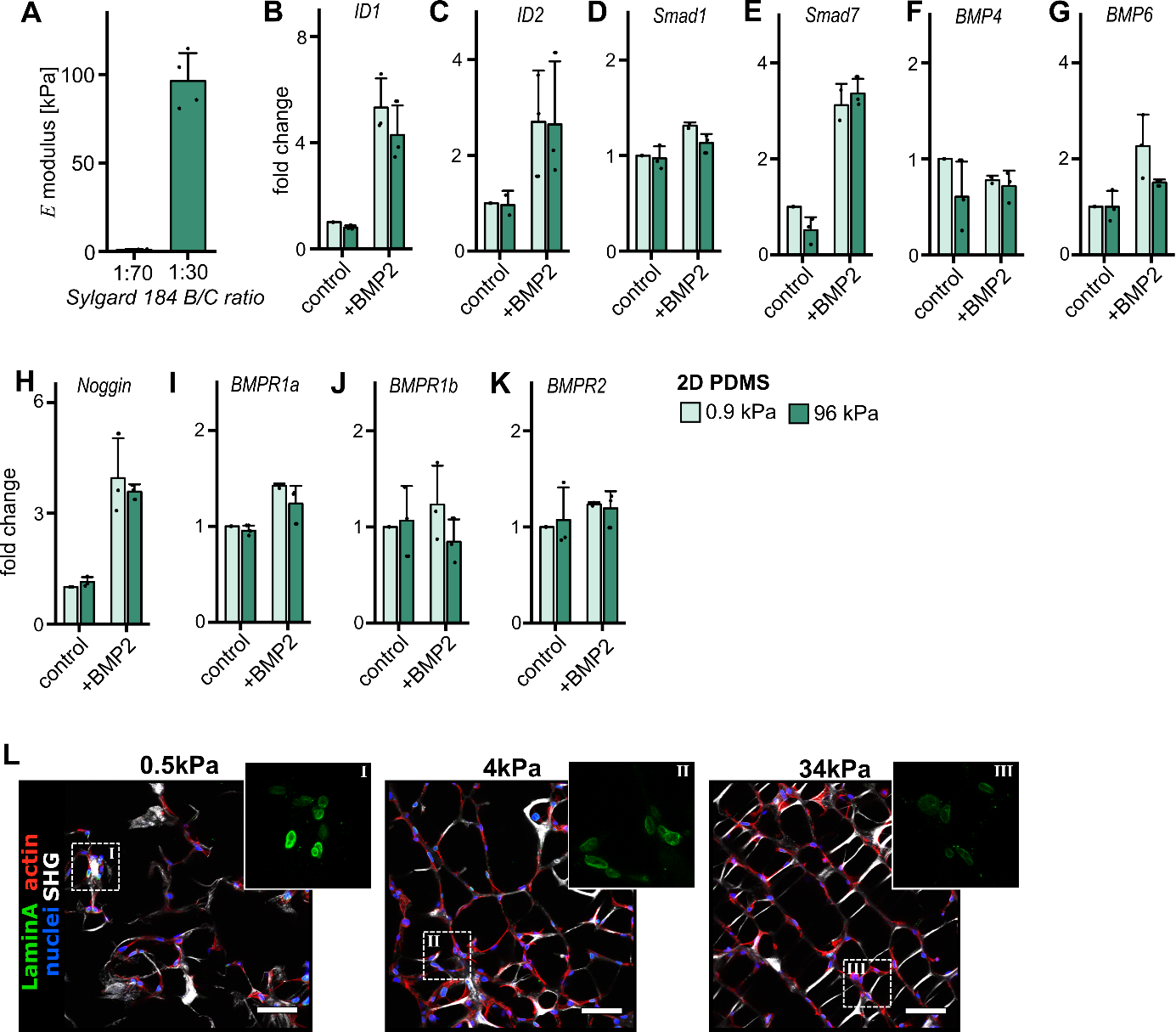
**Supplementary information**



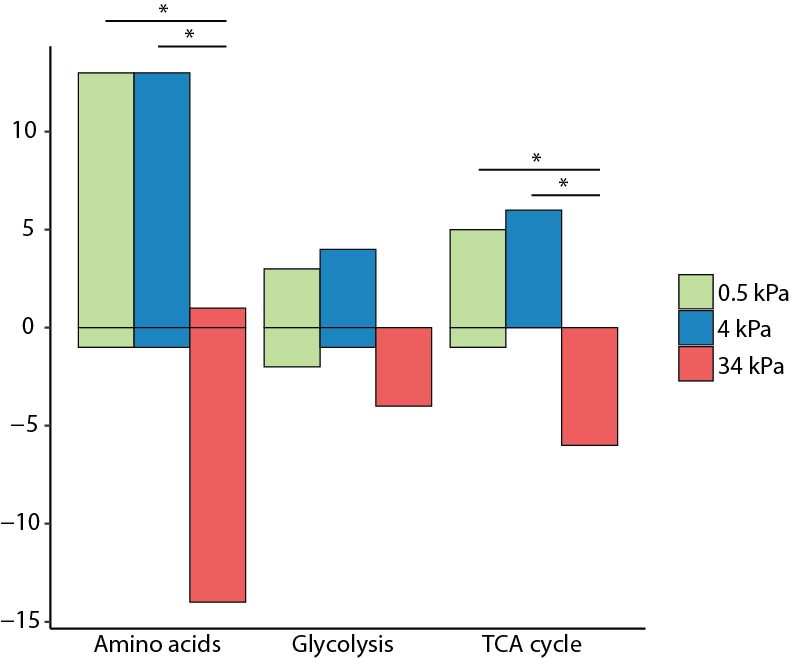
**Figure S1: (A)** Brightfield microscope image of a thin collagen dispersion smear visualized by differential interference contrast (DIC). Scale bar 50µm. **(B)** Quantification of the density of the wall-interconnecting struts (counts/mm³) using high-resolution second harmonic generation (SHG) images. **(C)** Quantification of the pore diameter (spanning distance between parallel walls) using high resolution SHG images. N > 50 of at least 2 independent samples. Statistics via the Mann–Whitney U test (two-sided). Significance levels are indicated as: \*\*\* p<0.001.



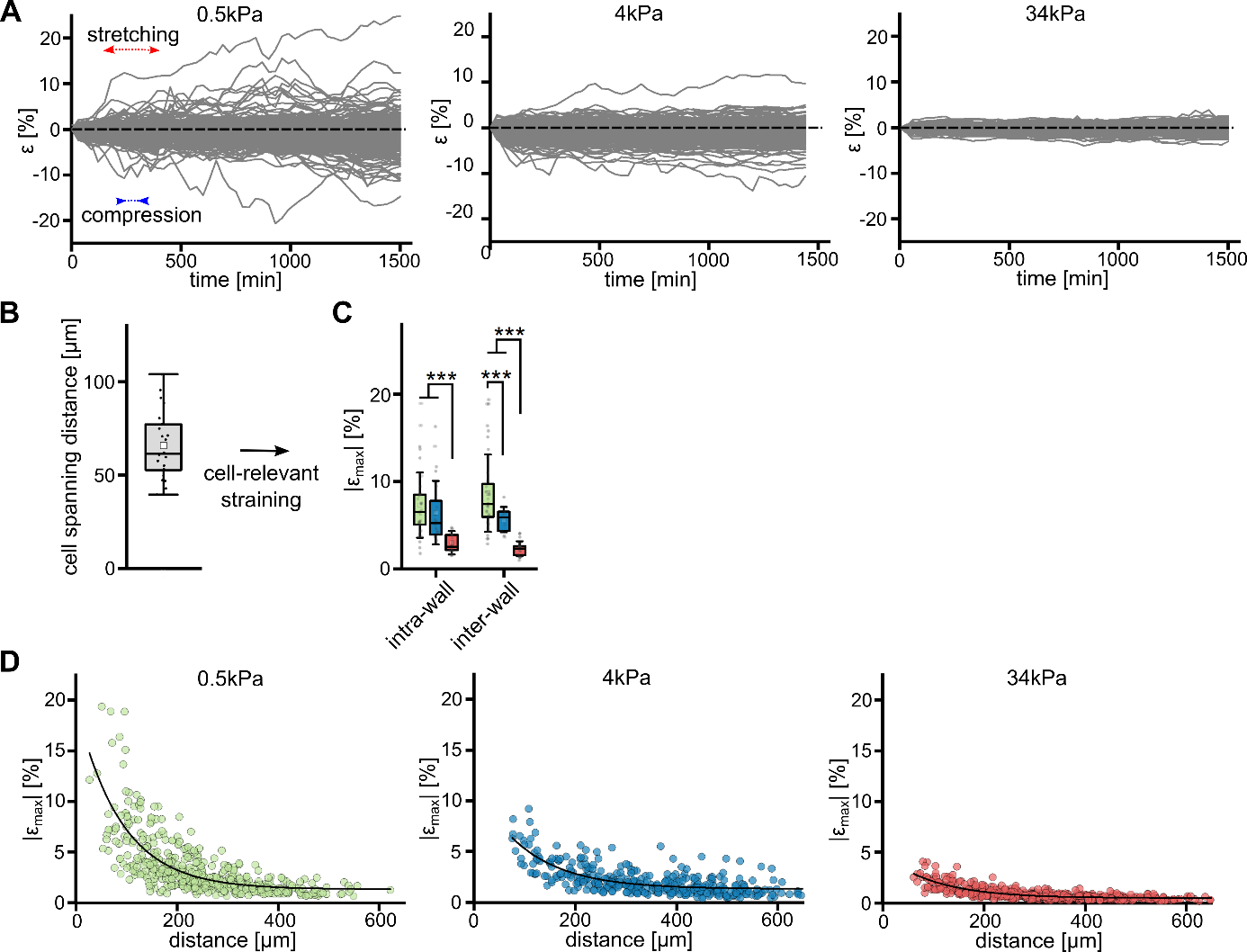
**Figure S2: (A)** Activity of soluble BMP over time in the presence of collagen scaffolds measured by luciferase reporter gene assay. N = 3. **(B)** to **(D)** Gene expression levels expressed as fold change to the unstimulated control of soft PAAm gel (1.8kPa, light grey). hFOBs were seeded on top collagen-coated PAAm gels and pre-cultivated for 48h prior to stimulation with 5nM BMP2 for 6h. N = 4. **(E)** to **(H)** Gene expression levels expressed as fold change to the unstimulated control of scaffold\_A (0.5kPa, green). Primary human MSCs were seeded into macroporous collagen scaffolds and stimulated with 5nM of rhBMP2 for 72h. N = 3 of three individual donors. Statistics via the Mann–Whitney U test (two-sided). Significance levels are indicated as: # p<0.1.



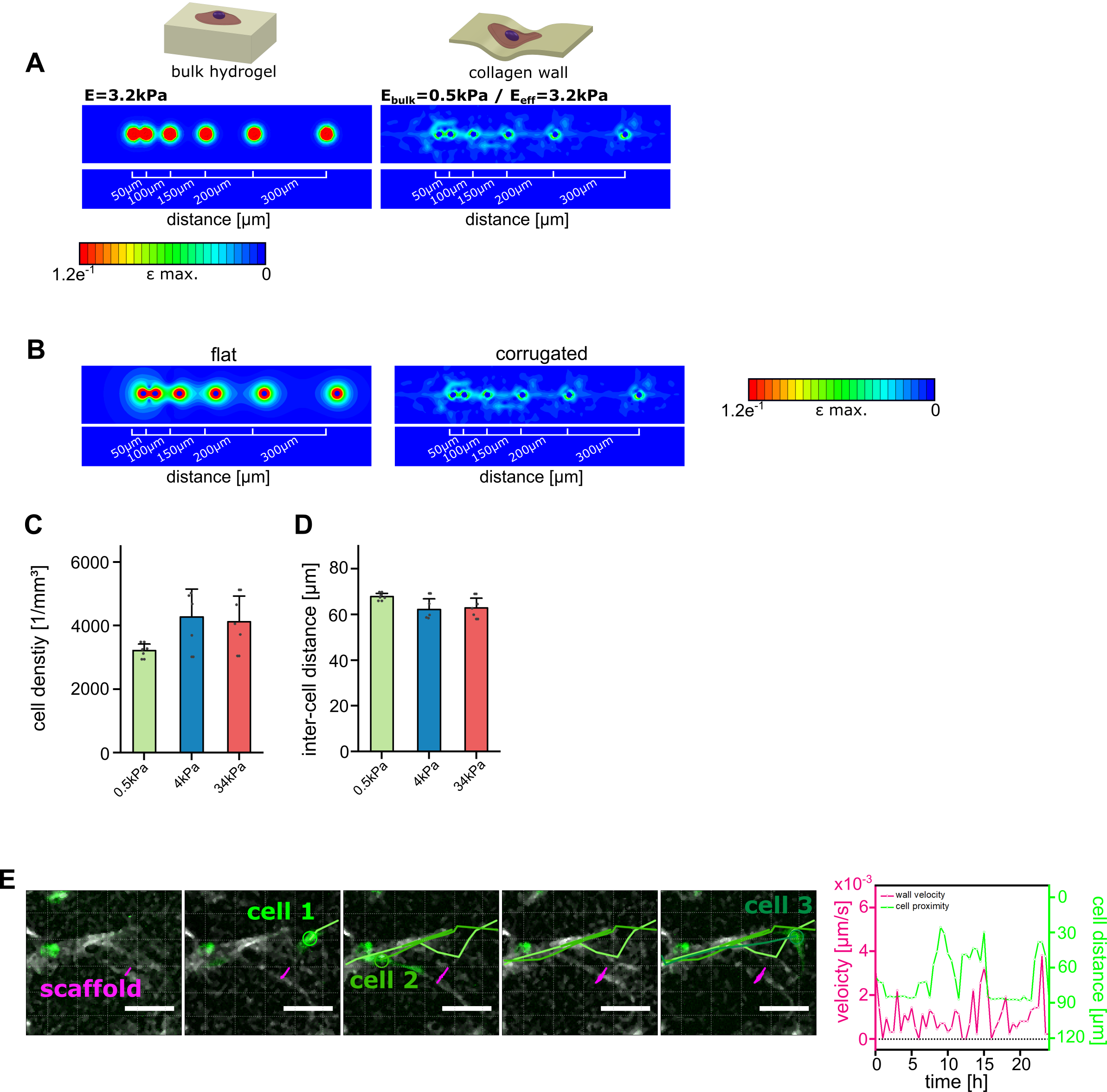
**Figure S3: (A)** Compression testing of cylindrical samples of Sylgard 184 PDMS created at different ratios of base and crosslinker (B/C). N=4. **(B) to (K)** Gene expression levels expressed as fold change to the unstimulated control of soft PDMS gel (1.8kPa, light green). hFOBs were seeded on top collagen-coated PAAm gels and pre-cultivated for 48h prior to stimulation with 5nM BMP2 for 6h. N = 3. **(L)** Confocal images of hFOBs cultured inside macroporous collagen scaffolds of varying stiffness. Cells were stained for LaminA (green), actin (red) and nuclei (blue). The scaffold was visualized by SHI (white). Dashed boxes outline zoom-in ROIs to visualize nuclear Lamin A levels. Scale bar 100µm.



**Figure S4:** Summary of the pathway perturbations occurring in different scaffolds as a sum of the number of individual metabolite changes in relation to the mean following unit scaling of normalized peak areas (in total and according to the subsets of metabolic pathways comprising glycolysis, TCA (tricarboxylic acid) cycle and amino acids. Metabolites with higher peak areas than the average for that metabolite are shown above zero and those with lower peak areas below. Y-axis: frequency of metabolites above or below individual metabolite mean average. Statistics via Student’s t-test. Significance levels are indicated as: \* p<0.05.



**Figure S5: (A)** Measured inter-wall biomaterial straining over time relative to reference length at time point 0. A negative value indicates compression, a positive stretching. **(B)** Cell spanning length in µm for hFOBs inside the scaffold biomaterial. **(C)** Intra- and inter-wall maximum scaffold straining ε for the cell-relevant length scale of a cell. Data points were filtered for the distance of the respective landmarks according to the range of the maximum cell spanning distance. **(D)** Inter-wall deformation expressed as maximum percentile strain ε as a function of the distance of the respective landmarks. Statistics via the Mann–Whitney U test (two-sided). Significance levels are indicated as: \*\*\* p < 0.001.



**Figure S6: (A)** Finite element simulation of material straining by thermal contraction of a circular cell element at different distances of 50, 100, 150, 200 and 300µm for soft bulk elastic hydrogels and soft scaffold walls. **(B)** Finite element simulation of material straining by thermal contraction of a circular cell element at different distances of 50, 100, 150, 200 and 300µm for soft scaffold walls either without (flat) or with (corrugated) an intrinsic corrugation. **(C)** cell density of hFOBs after 2 days of culture for the three different stiffnesses. (**D)** corresponding inter-cell distance based on cell density assuming an equal distribution of cells. **(E)** time lapse imaging and quantification of cell migration (cell=green) inside a soft collagen scaffold (grey=collagen) indicating migration-dependent deformation of the underlying biomaterial. Scale bar 20µm.

**Supplementary Table 1:** Summary of all primer sequences used for gene expression analysis. HPRT (grey highlight) was used as a reference housekeeping gene.

|  |  |  |
| --- | --- | --- |
| **Gene name** | **Forward sequence (5’ – 3’)** | **Reverse sequence (5’ – 3’)** |
| ID1 | GCTGCTCTACGACATGAACG | CCAACTGAAGGTCCCTGATG |
| ID2 | GTGGCTGAATAAGCGGTGTT | TGTCCTCCTTGTGAAATGGTT |
| BMP4 | CCACGAAGAACATCTGGAGAAC | ATACGGTGGAAGCCCCTTT |
| BMP6 | GCAGACCTTGGTTCACCTTATG | AGAATGTGTGTCCCCAGCA |
| Noggin | GCCAGCACTATCTCCACATCC | GGGTGTTCGATGAGGTCCAC |
| Smad1 | CAACAGAGGAGATGTTCAGGC | AGTGAAACCATCCACCAACAC |
| Smad7 | CCCATCACCTTAGCCGACTC | TTGGGAATCTGAAAGCCCCC |
| BMPR1a | TTGGGAATCTGAAAGCCCCC | ACGACGTCTGCTTGAGATGC |
| BMPR1b | CCTGGAGAATCCCTGAGAGAC | AGTCCTTTGGACCAGCAGAG |
| BMPR2 | GTTGGAGCTGATTGGCCGAG | TTTACAGCAACTGGACGCTC |
| Runx2 | CTCCTACCTGAGCCAGATGA | CGGGGTGTAAGTAAAGGTGG |
| Sox9 | GGAGACTTCTGAACGAGAGCG | CCGTTCTTCACCGACTTCCTC |
| PPARG | TGCAGTGGGGATGTCTCATA | CAGCGGGAAGGACTTTATGT |
| COL2A1 | CTGGAAAAGATGGTCCCAAA | CAGGGAATCCTCTCTCACCA |
| ACAN | GGGTTTTCGTGACTCTGAGG | ATGGGGTCGATGAAATAGCA |
| HPRT | TATGGACAGGACTGAACGTC | TGATGTAATCCAGCAGGTCA |

**Supplementary Table 2:** List of metabolite derivatives and their biological groups used for reference search. AA: Amino acids. PPP: Pentose phosphate pathway. TCA: Tricarboxylic acid cycle. TMS: Trimethylsilyl derivatives. MeOX: Methoxyamine hydrochloride.

|  |  |  |  |
| --- | --- | --- | --- |
| **Group** | **Metabolite** | **Abbreviation** | **Detected as** |
| AA | Alanine | Ala | 3TMS |
|  |  |  | 2TMS |
| AA | Asparagine | Asn | 2TMS |
| AA | Aspartic acid | Asp | 2TMS |
|  |  |  | 3TMS |
| AA | Cysteine | Cys | 3TMS |
| AA | Glycine | Gly | 2TMS |
|  |  |  | 3TMS |
| AA | Isoleucine | Ile | 1TMS |
|  |  |  | 2TMS |
| AA | Leucine | Leu | 1TMS |
|  |  |  | 2TMS |
| AA | Lysine | Lys | 3TMS |
| AA | Methionine | Met | 1TMS |
|  |  |  | 2TMS |
| AA | Phenylalanine | Phe | 1TMS |
|  |  |  | 2TMS |
| AA | Proline | Pro | 1TMS |
|  |  |  | 2TMS |
| AA | Serine | Ser | 2TMS |
|  |  |  | 3TMS |
|  |  |  | 4TMS |
| AA | Threonine | Thr | 2TMS |
|  |  |  | 3TMS |
| AA | Tryptophan | Trp | 2TMS |
| AA | Tyrosine | Tyr | 3TMS |
| AA | Valine | Val | 1TMS |
|  |  |  | 2TMS |
| Glycerol | Dihydroxyacetone phosphate | DHAP | 1MeOX 3TMS |
| Glycerol | Glycerol | Glyc | 3TMS |
| Glycerol | Glycerol-3-phosphate | Glyc3P | 4TMS |
| Glycolysis | Fructose-6-phosphate | F6P | 1MeOX 6TMS |
| Glycolysis | Glucose-6-phosphate | G6P | 1MeOX 6TMS |
| Glycolysis | Glyceric acid-3-phosphate | GA3P | 4TMS |
| Glycolysis | Lactic acid | Lac | 2TMS |
| Glycolysis | Phosphoenolpyruvic acid | PEP | 3TMS |
| Glycolysis | Pyruvic acid | Pyr | 1MeOX 1TMS |
| Nucleobase | Adenine | Adenine | 2TMS |
| Nucleobase | Uracil | Uracil | 2TMS |
| Nucleosid | Adenosine | Adenosine | 3TMS |
|  |  |  | 4TMS |
| Nucleosid | Cytosine | Cytosine | 2TMS |
| Others | Butanoic acid, 3-hydroxy- | But3h | 2TMS |
| Others | Butanoic acid, 4-amino- | But4am | 3TMS |
| Others | Erythritol | Ery | 4TMS |
| Others | Glutaric acid | Glut | 2TMS |
| Others | Glyceric acid | Glyc | 3TMS |
| PPP | Ribose-5-phosphate | R5P | 1MeOX 5TMS |
| PPP | Ribose | Ribose | 1MeOX 4TMS |
| TCA | Citric acid | Cit | 4TMS |
| TCA | Fumaric acid | Fum | 2TMS |
| TCA | Glutaric acid, 2-hydroxy- | 2HG | 3TMS |
| TCA | Glutaric acid, 2-oxo- | aKG | 1MeOX 2TMS |
| TCA | Malic acid | Mal | 3TMS |
| TCA | Succinic acid | Suc | 2TMS |

**Supplementary Table 3:** Technical variation during gas chromatography mass spectrometry (GC-MS) runs. RSD: Relative standard deviation. QC: quality control.

|  |  |  |
| --- | --- | --- |
| **Parameter** | **QC** | **Samples** |
| RSD of internal standard | 7% | 17% |
| Median RSD | 18% | Soft 25%  Medium 41%  Stiff 33% |