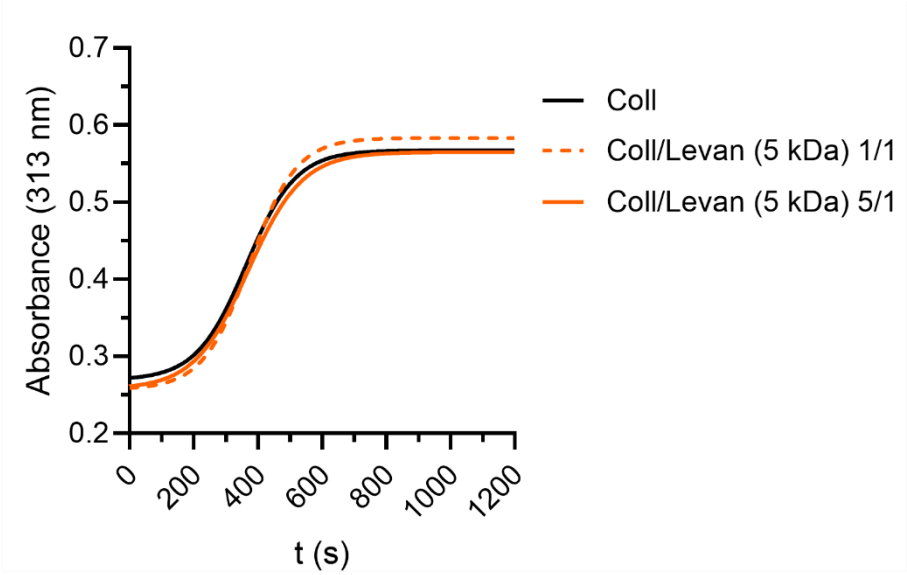
Supporting Information

Self-assembled biomimetic microenvironments with sulfated levan promote kidney epithelial cell growth and reduce inflammatory cytokine release

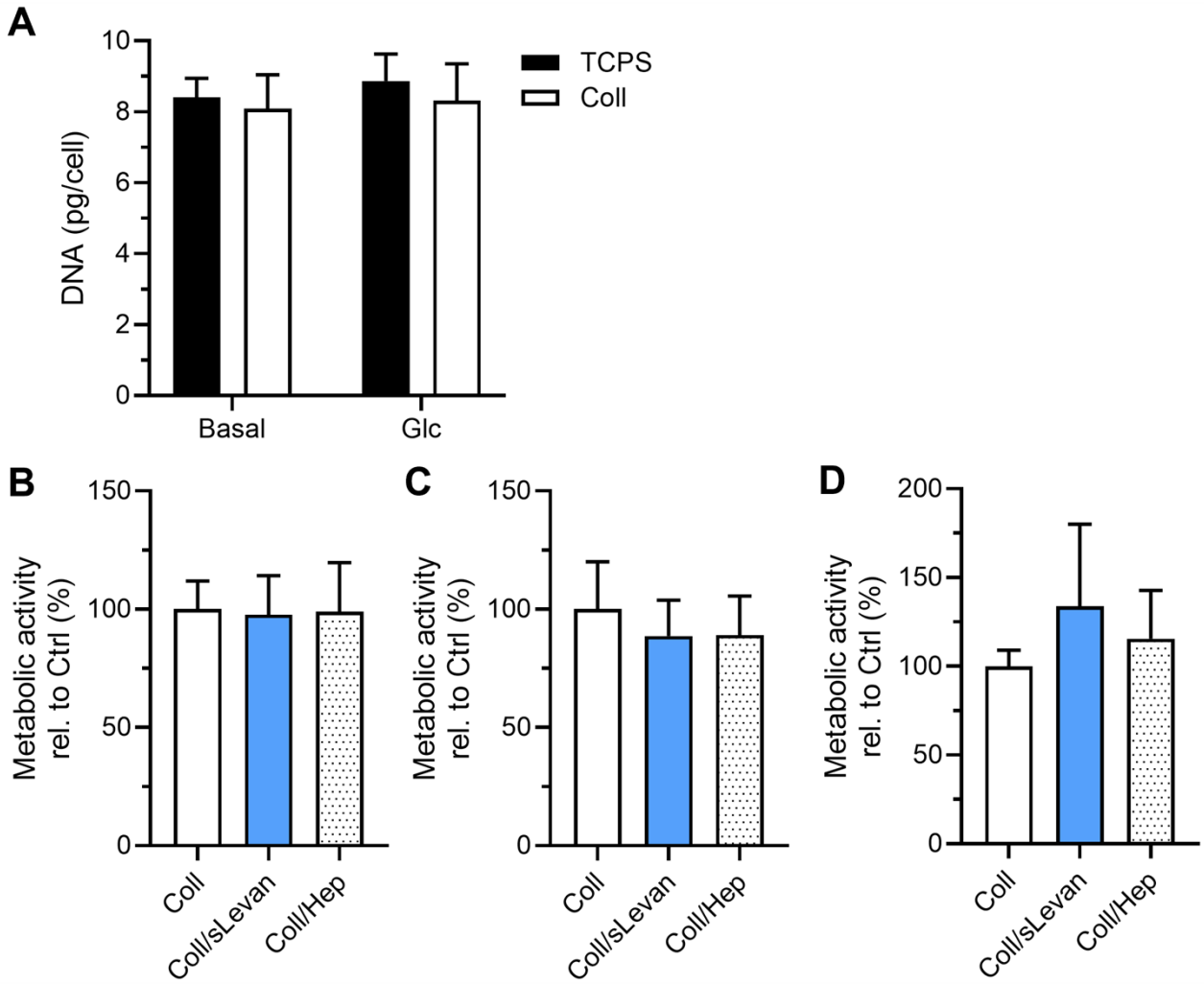
Cell culture of HUVECs, pericytes, HEK-293 cells

Human umbilical vein endothelial cells (HUVEC), human pericytes from placenta (hPC-PL), both from PromoCell (Heidelberg, Germany), and human embryonic kidney cells (HEK-293) from ATCC ((Manassas, VA, USA) were cultivated in endothelial cell basal medium with 100 U/mL penicillin and 0.1 mg/mL streptomycin (PromoCell, Heidelberg, Germany), pericyte basal medium 2 with 100 U/mL penicillin and 0.1 mg/mL streptomycin (PromoCell, Heidelberg, Germany) or high glucose DMEM with 10 % fetal calf serum, 100 U/mL penicillin and 0.1 mg/mL streptomycin (PAN Biotech, Aidenbach, Germany), respectively, at 37 °C under a humidified 95 % to 5 % (v/v) mixture of air and CO_2.

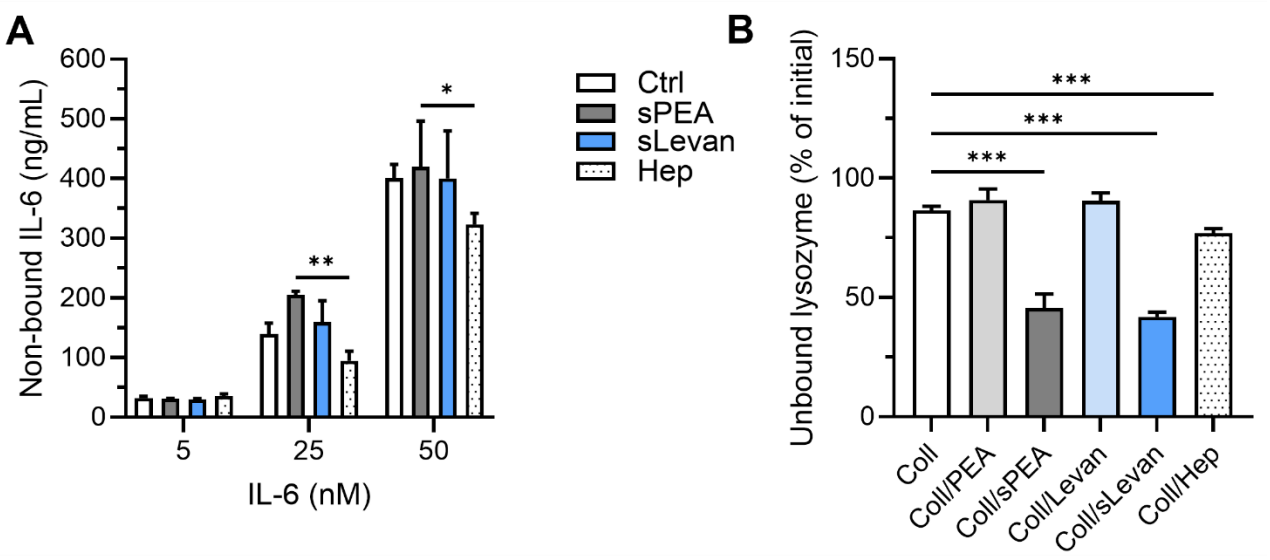
To assess the influence of biomimetic ECMs on cellular metabolic activity, 40,000 cells per well of each cell type were seeded into Coll, Coll/sLevan and Coll/Hep pre-coated 48-well plates and cultivated for 48 hours before quantifying cell viability by means of the Celltiter-Blue Cell Viability Assay (Promega Corporation, Germany). For this purpose, 30 µL of the Celltiter-Blue reagent were applied to each well and incubated for 150 minutes at 37 °C and 5 % CO_2. Fluorescence intensity was detected using a Spark multimode microplate reader (Tecan, Crailsheim, Germany) with λ_{ex} = 560 nm, λ_{em} = 590 nm. Each material type was tested with a sample size of n = 7 per condition.



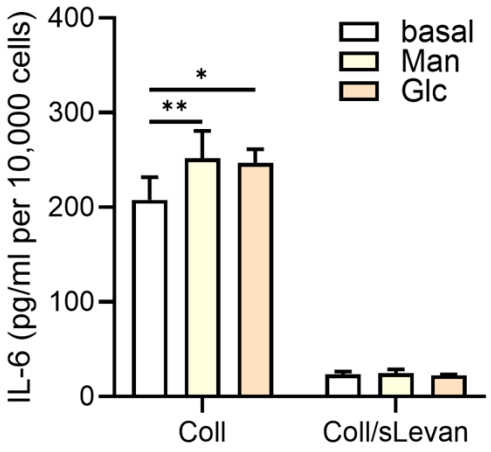
SI Fig. 1 Kinetics of biomaterial self-assembly in the presence of low-molecular weight levan. The in-vitro fibrillogenesis of collagen type I was monitored by turbidity measurements at 313 nm in the presence of different collagen-to-Levan (5 kDa) mass ratios (1/1 to 5/1). Each curve represents the mean of three independent measurements.



SI Fig. 2 DNA per cell values of HK-2 cells cultured on TCPS or Coll for 48 hours under basal or high-glucose conditions and effects of microenvironments on different cell types. (A) DNA content of HK-2 cells was quantified using the PicoGreen assay with λ-DNA as calibration standard and correlated to cell numbers via a calibration curve from defined HK-2 cell counts. Statistical analysis was performed using two-way ANOVA. (B) HUVEC ~~(A)~~, human (C) pericytes ~~(B)~~ and (D) HEK-293 cells were cultured for 48 hours on microenvironments incorporating sLevan or Hep. Coll without polysaccharides served as control. The cellular metabolic activity was measured using the Celltiter Blue Assay. Statistical analysis was performed using one-way ANOVA.



SI Fig. 3 Binding profile of IL-6 to immobilized polysaccharides and lysozyme to biomimetic microenvironments. (A). Polysaccharides (sPEA, sLevan, and Hep) were covalently immobilized on polystyrene surfaces and subsequently incubated with IL-6. Unbound IL-6 was quantified by ELISA, with BSA-coated wells serving as negative controls. Two-way ANOVA: * $p < 0.05$ ** $p < 0.01$. (B) Binding of rhodamine B-labeled lysozyme to self-assembled biomimetic microenvironments. Samples were incubated with lysozyme, and unbound protein in the supernatant was quantified by fluorescence. Data are presented as unbound lysozyme (% of initial). One-way ANOVA: *** $p < 0.001$ vs Coll.



SI Fig. 4 IL-6 secretion of HK-2 cells under osmotic and hyperglycemic conditions. IL-6 secretion of HK-2 cells was quantified by ELISA after 48 hours of culture on collagen-based microenvironments under basal, osmotic (Man), and hyperglycemic (Glc) conditions. IL-6 concentrations in culture supernatants were normalized to cell number. Two-way ANOVA: * $p < 0.05$ ** $p < 0.01$.