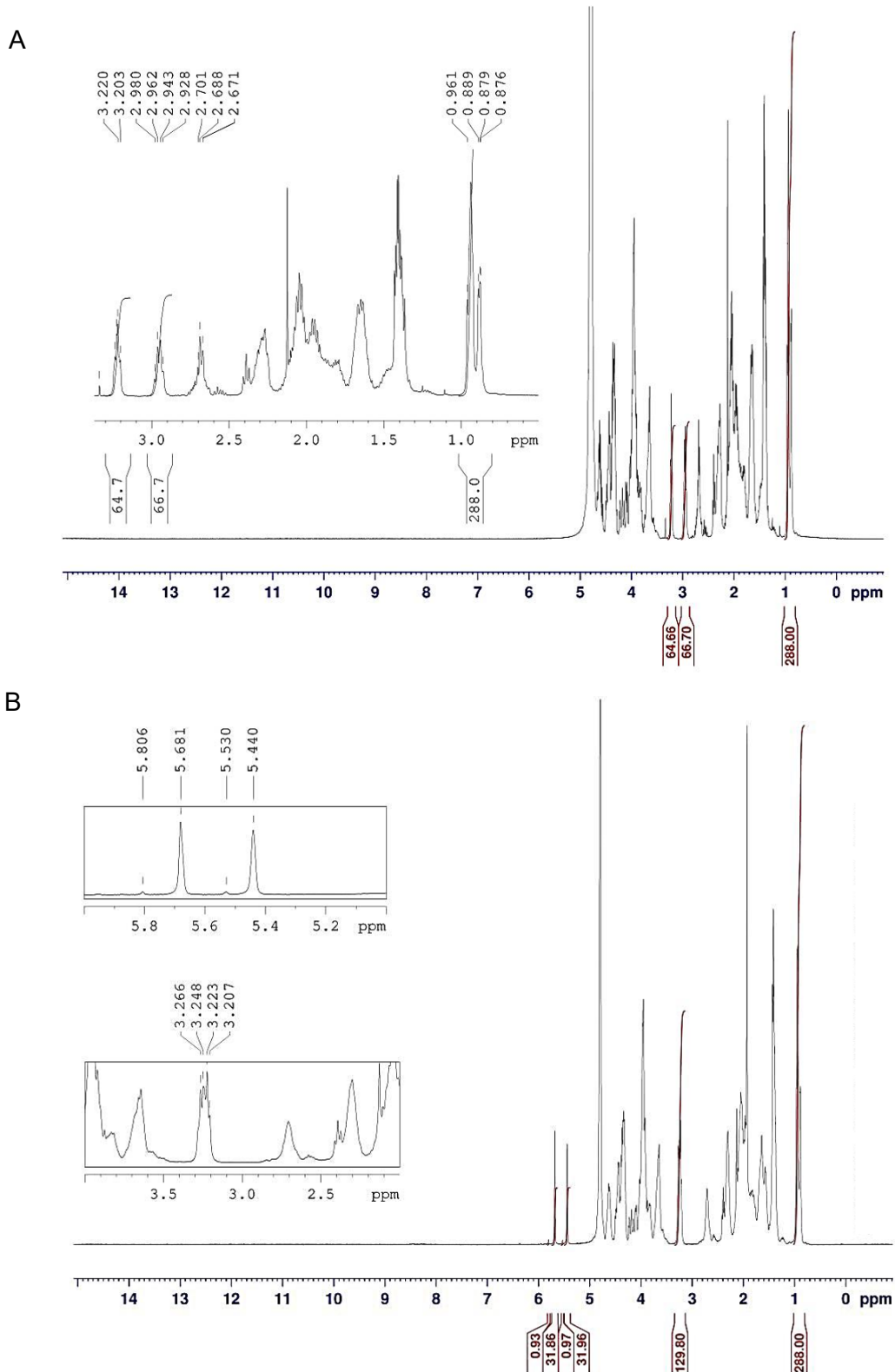


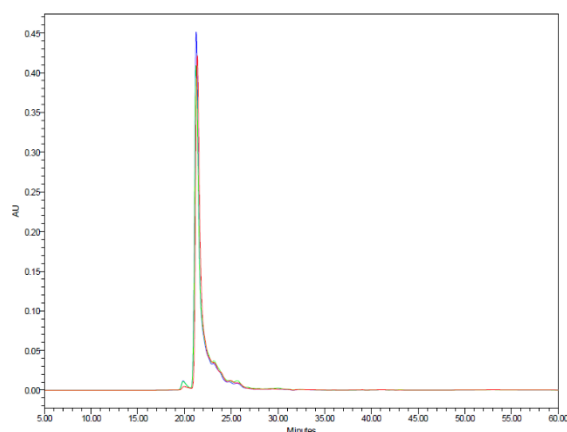
**Supplemental information**

**Methacrylated human recombinant collagen peptide  
as a hydrogel for manipulating and monitoring  
stiffness-related cardiac cell behavior**

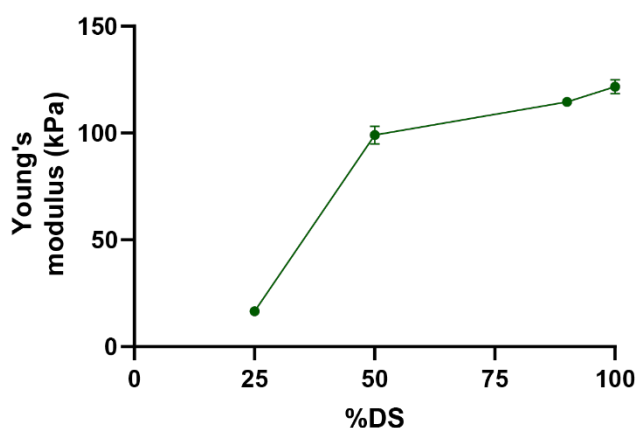
**Dylan Mostert, Ignasi Jorba, Bart G.W. Groenen, Robert Passier, Marie-José T.H. Goumans, Huibert A. van Boxtel, Nicholas A. Kurniawan, Carlijn V.C. Bouten, and Leda Klouda**



**Figure S1:** The  $^1\text{H}$ -NMR spectrum of RCPHC1 (A, upper plot) and RCPHC1-MA DS100% (B, lower plot) recorded on a Bruker 400MHz spectrometer in  $\text{D}_2\text{O}$ , at  $25^\circ\text{C}$ , 15mg/ml. The vinylic protons of N-terminal glycine coupled methacrylamide moieties are visible as very small peaks at 5.806 and 5.530 ppm, 3% of the combined vinylic protons integral. Related to STAR Methods.



**Figure S2:** Molecular weight distribution overlay of native RCPHC1 (red) and two batches RCPHC1-MA DS100% (green and blue). No significant adjustments in molecular weight distribution were observed for methacrylated RCPHC1. Related to STAR Methods.



**Figure S3:** Standard exposed 10% (w/v) RCPHC1-MA hydrogel DS-dependent mechanical strength. RCPHC1-MA hydrogels were fabricated with 0.067 wt% LAP and exposed at 405 nm at 10 mW/cm<sup>2</sup> for 60 seconds, monitored for at least 20 minutes. The hydrogels were incubated in PBS at 37°C for two days before measuring the compressive modulus. All data is presented as mean  $\pm$  SEM (n=3 per condition). Related to Figure 2.

**Table S1:** Composition of TDI medium. Related to STAR Methods.

For 1000 mL

- 961 mL Distilled water
- 10 mL Chemical defined lipid concentrate, Thermo Fisher, 11905031
- 10 mL GlutaMAX, Thermo Fisher, 35050038
- 10 mL Ascorbic acid, Sigma-Aldrich, A8960-5G
- 5 mL P/S, Thermo Fisher, 15070063
- 5 mL Sodium Pyruvate, Thermo Fisher
- 1 mL ITS-X, Thermo Fisher, 51500056
- 1 mL Trace elements A, Cellgro, 25-021-CI
- 1 mL Trace elements B, Cellgro, 25-022-CI
- 1 mL Trace elements C, Cellgro, 25-023-CI
- 0,39 µL αMTG, Sigma-Aldrich, M6154-25ml
- 8,3 gr DMEM, Sigma-Aldrich, D5030-10x1L
- 2,73 gr D(+)-Glucose, Milipore, 1083371000
- 0,626 gr Taurine, Sigma-Aldrich, T8691-100G
- 0,15 gr Creatine monohydrate, Sigma-Aldrich, C3630-100G
- 0,10 gr L-Carnitine hydrochlorine, Sigma-Aldrich, C0283-5G
- 0,024 gr Sodium 3-hydroxybutyrate, Sigma-Aldrich, 54965-10G
- 0,011 gr Phenol Red, Sigma-Aldrich, P3532-5G

Add to TDI medium less than one week prior to usage:

- 100ng/mL T3, Sigma-Aldrich, T6397-100G
- 100ng/mL IGF-I human, Sigma-Aldrich, I1271
- 1µg/mL Dexamethasone, Tocris, 1126/100

**Table S2:** List of all used primary- and secondary antibodies and dyes. Related to STAR Methods.

Antigen	Source	Cat.No	Isotype	Label	Species	Dilution
Vimentin	Abcam	Ab20346	IgM	-	Mouse	1/200
Mouse IgM	Molecular Probes	A21042	-	Alexa Fluor 488	Goat	1/200
Phalloidin	Thermo-Fisher	-	-	Alexa Fluor 647	-	1/400
DAPI	Sigma-Aldrich	-	-	-	-	1/500