Supplementary Information

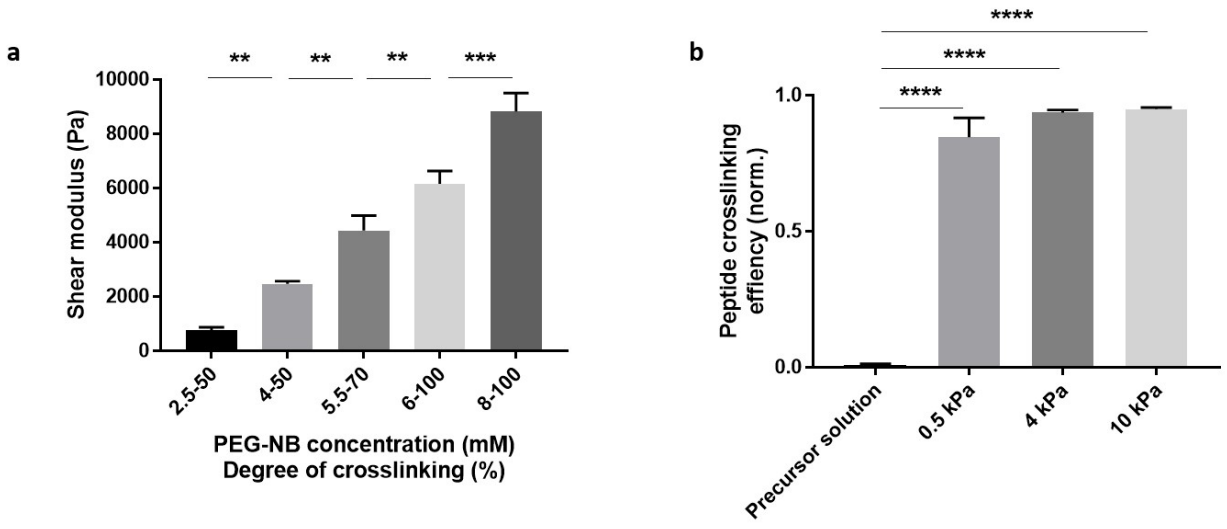

Figure S1. PEG hydrogel characterization revealed precise control of (**a**) substrate stiffness, measured as shear modulus, and (**b**) peptide concentration. ** p<0.01 *** p<0.001 **** p<0.0001, one-way ANOVA. Each bar = 3 replicate gels.

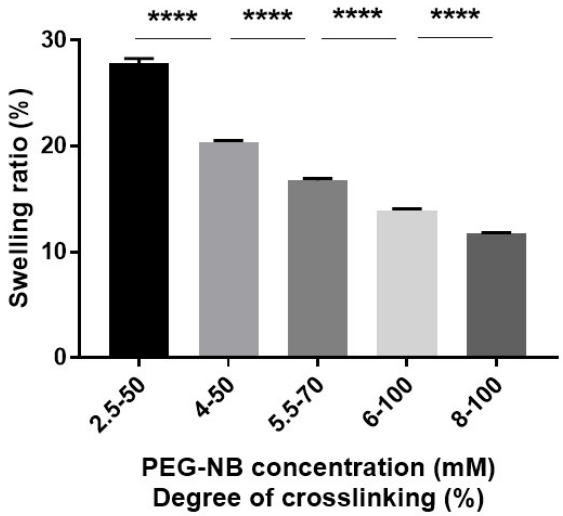

Figure S2. PEG hydrogel swelling ratio varied with input concentrations. **** p<0.0001, one-way ANOVA. Each bar = 3 replicate gels.

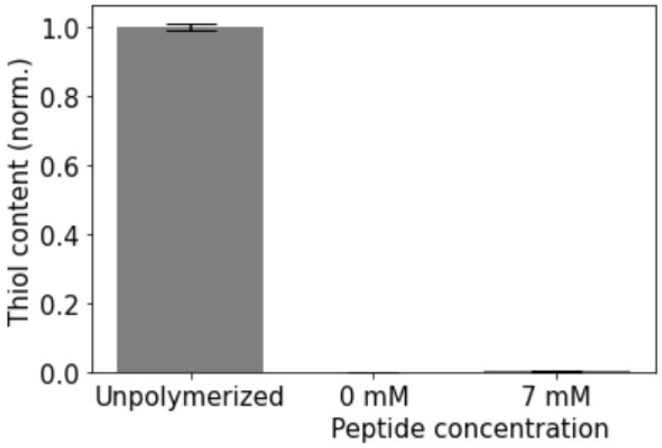

Figure S3. PEG hydrogel thiol content varied with input peptide concentration. Each bar = 3 replicate gels.

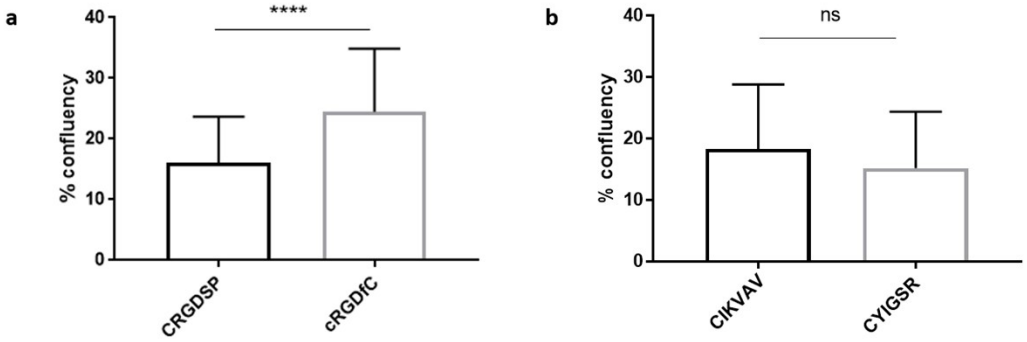

Figure S4. Confluency quantification of iPSC-CPCs cultured on PEG hydrogels presenting (**a**) different primary peptides, and (**b**) different secondary peptides. ns: no significance, **** p<0.0001, Student t-test. Each bar = 50 gels.

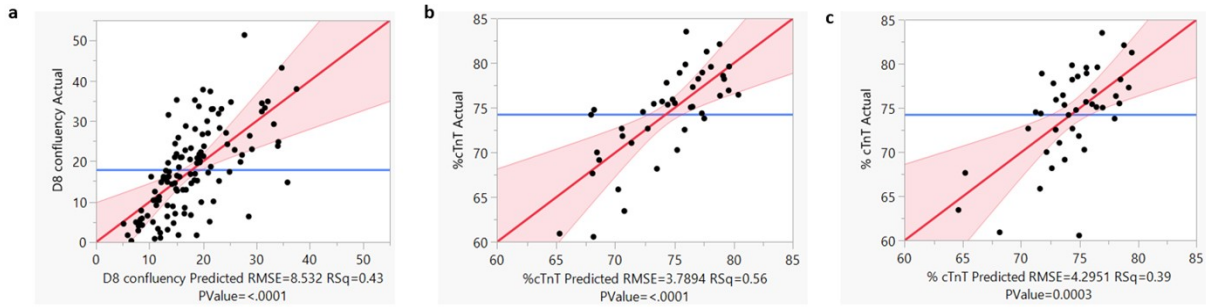

Figure S5. Actual by predicted plots of standard least squares models of the effect of (**a**) substrate parameters on the degree of cell confluency (Fig. 2e), (**b**) substrate parameters on the level of cTnT expression (Fig. 4b), and (**c**) confluency levels and cell number on the level of cTnT expression (Fig. 4c).

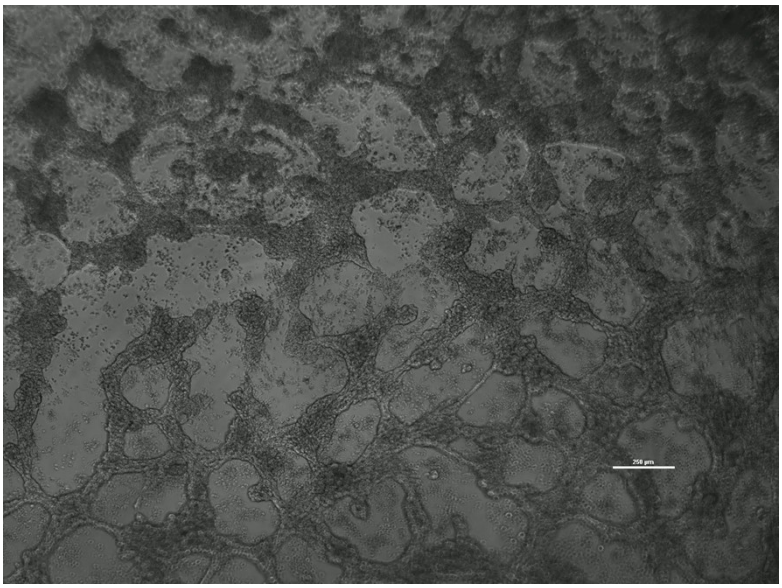

Figure S6. Brightfield video recording of iPSC-CMs cultured on PEG hydrogel (3.5C 0.5). Scale = 250 µm.

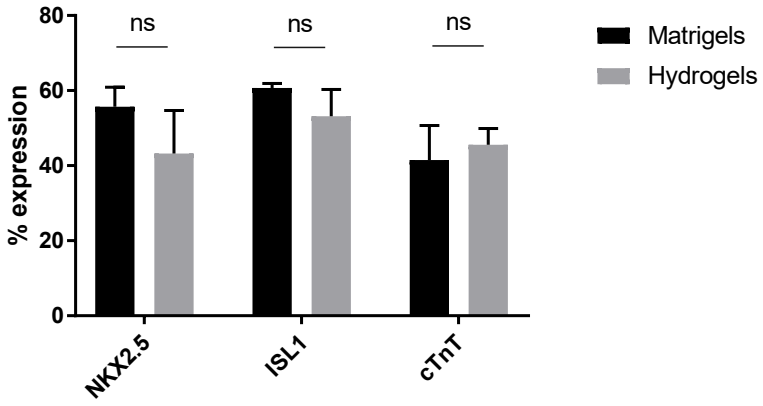

Figure S7. Flow cytometry quantification of CPC markers (NKX2.5, ISL1) and CM markers (cTnT) on Matrigel and PEG hydrogels. ns = no significance, one-way ANOVA. Each bar = 6 gels (Matrigel) or 9 gels (PEG hydrogels).

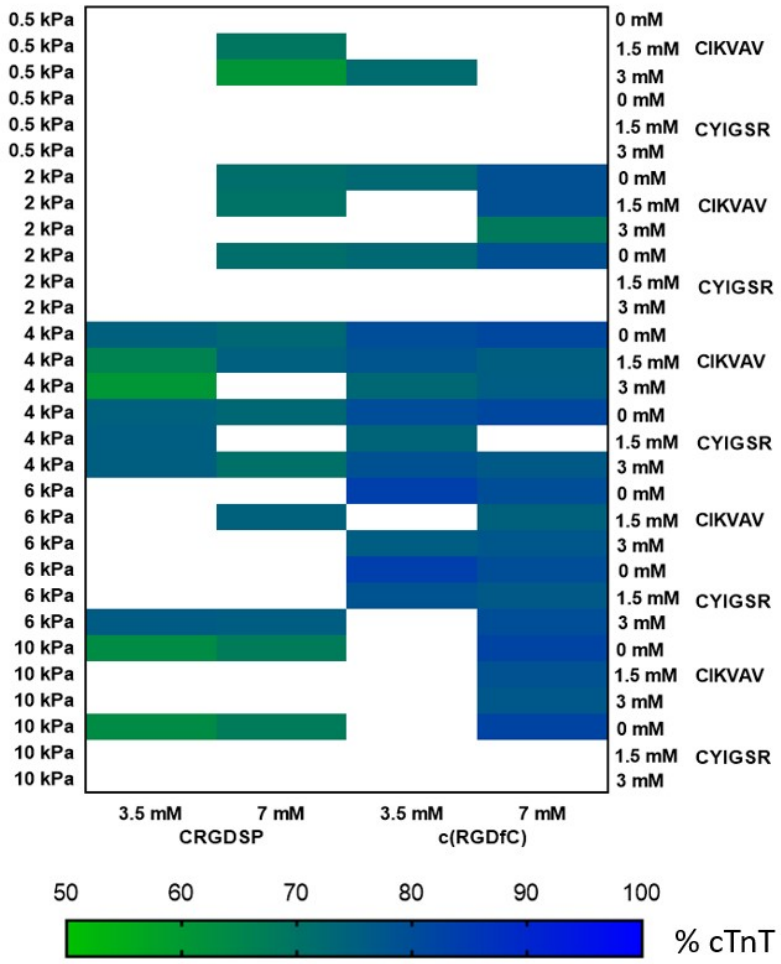

Figure S8. Quantitative heat map of cTnT-expressing cell levels after culture on varying PEG hydrogel formulations. Each box = 3 replicate gels, blank box = not tested.

Synthetic peptide	Primary/Secondary	Abbreviation	ECM protein
CRGDSP	Primary	L	Fibronectin
head-to-tail cyclized RGDfC	Primary	C	Fibronectin
CIKVAV	Secondary	I	Laminin
CYIGSR	Secondary	Y	Laminin

Table S1. Description of synthetic peptides incorporated in PEG hydrogels.

Primary peptide	Concentration (mM)	Secondary peptide	Concentration (mM)	Shear modulus (kPa)
L	7	-	-	0.5
L	7	-	-	2
L	3.5	-	-	4
L	7	-	-	4
L	3.5	-	-	10
L	7	-	-	10
C	3.5	-	-	0.5
C	3.5	-	-	2
C	7	-	-	2
C	3.5	-	-	4
C	7	-	-	4
C	3.5	-	-	6
C	7	-	-	6
C	7	-	-	10
L	3.5	I	1.5	4
L	3.5	I	3	4
L	7	I	1.5	0.5
L	7	I	3	0.5
L	7	I	1.5	2
L	7	I	3	2
L	7	I	1.5	4
L	7	I	1.5	6
L	7	I	3	6
L	3.5	Y	1.5	4
L	3.5	Y	3	4
L	3.5	Y	3	6
L	7	Y	3	4
L	7	Y	3	6

C	3.5	I	3	0.5
C	3.5	I	1.5	4
C	3.5	I	3	4
C	3.5	I	3	6
C	7	I	3	0.5
C	7	I	1.5	2
C	7	I	3	2
C	7	I	1.5	4
C	7	I	3	4
C	7	I	1.5	6
C	7	I	3	6
C	7	I	1.5	10
C	7	I	3	10
C	7	Y	3	0.5
C	3.5	Y	1.5	4
C	3.5	Y	3	4
C	3.5	Y	1.5	6
C	7	Y	3	4
C	7	Y	1.5	6
C	7	Y	3	6

Table S2. PEG hydrogel formulations tested to assess iPSC-CM differentiation efficiency (Fig. 3a).

Primary peptide	Concentration (mM)	Secondary peptide	Concentration (mM)	Shear modulus (kPa)
L	3.5	-	-	4
C	3.5	-	-	0.5
C	7	Y	3	6

Table S3. PEG hydrogel formulations tested to study CPC and CM markers (Fig. 3b).

Primary peptide	Concentration (mM)	Secondary peptide	Concentration (mM)	Shear modulus (kPa)
L	7	-	-	2
L	7	-	-	4
C	3.5	-	-	0.5
C	7	-	-	0.5
C	7	-	-	6
C	7	-	-	10
L	7	I	1.5	6
C	3.5	I	3	0.5
C	3.5	I	3	4
C	3.5	Y	3	0.5
C	7	Y	3	4

Table S4. PEG hydrogel formulations tested to assess ability to rescue iPSC-CM differentiation efficiency (Fig. 3d).