Poly(vinyl alcohol-co-itaconic acid) hydrogels grafted with several designed peptides for human pluripotent stem cell culture and differentiation into cardiomyocytes

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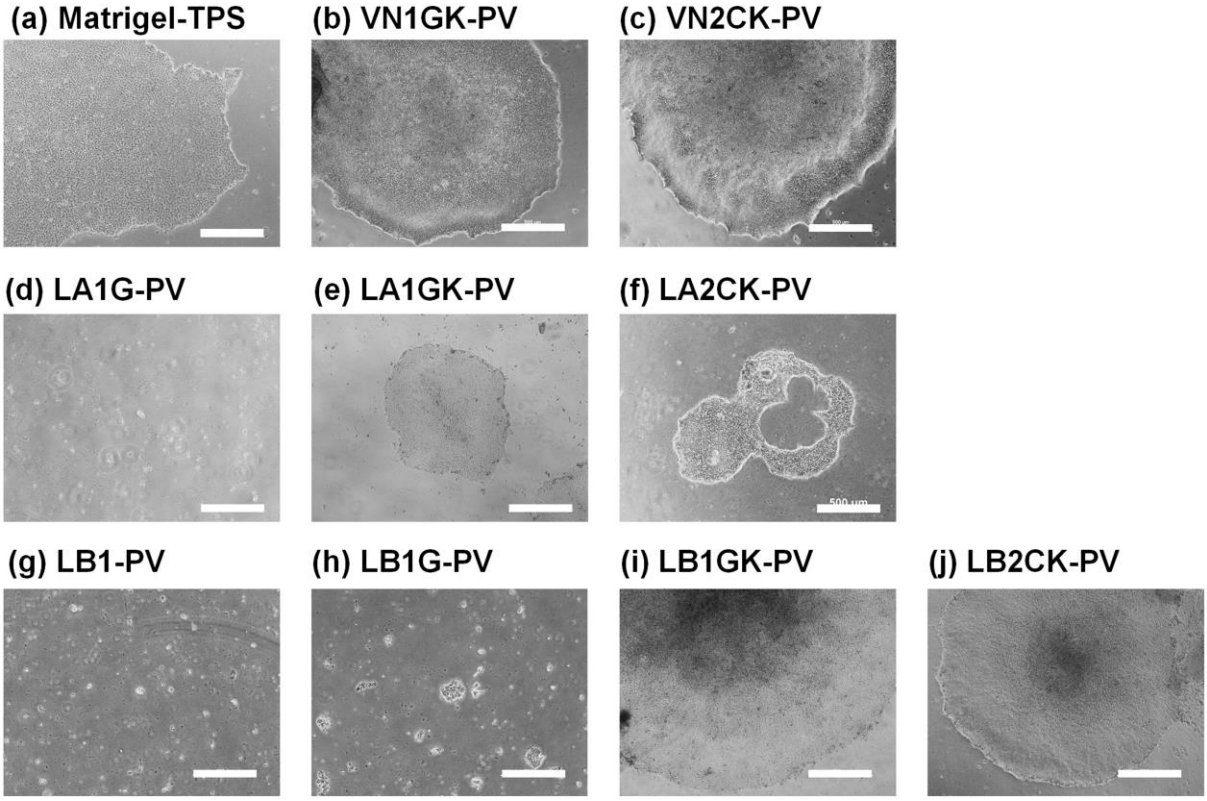
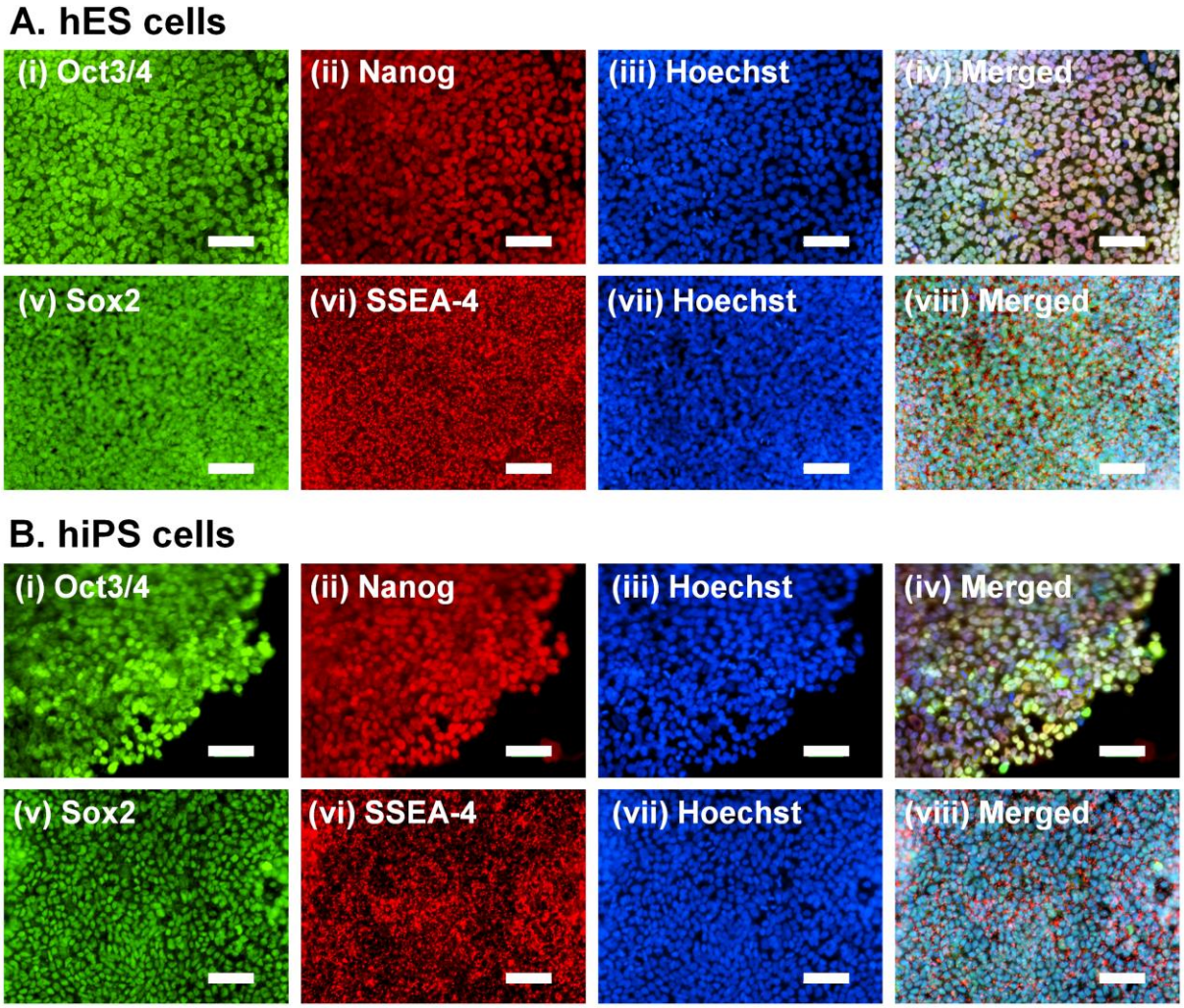
Supplementary Information

Supplementary Table 1 Materials used in this study.

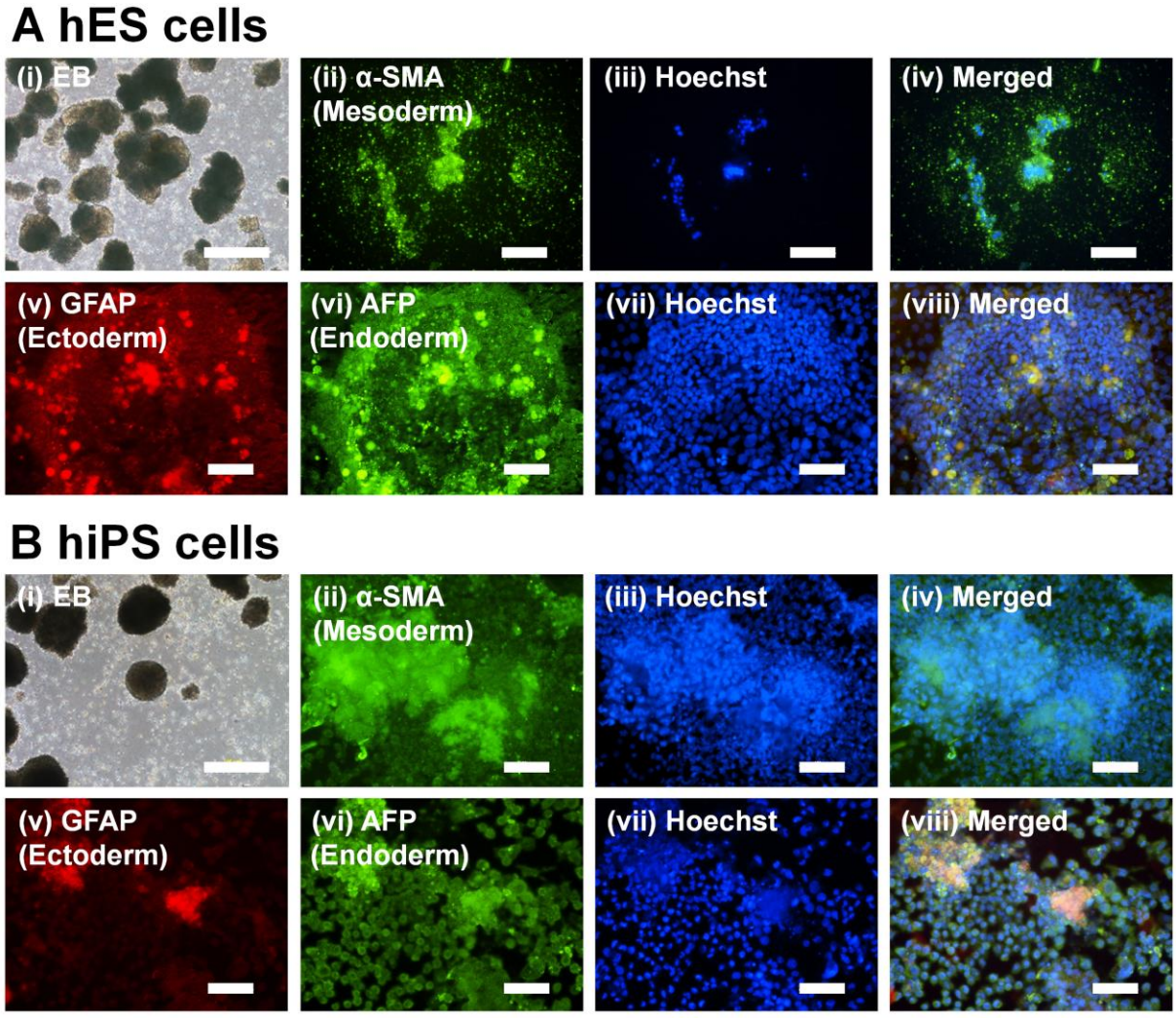
Materials	Abbreviation	Catalog No.	Company
		ECM	
Matrigel	Matrigel	#356230	Corning (Corning, NY, USA)
Recombinant vitronectin	rVN	A14700	Thermo Fisher Scientific (Waltham, MA, USA)
		Cell culture dishes	
6-well polystyrene plate	TCP	#353046	Corning (Corning, NY, USA)
		Chemicals & Polymer	
Dispase II	Dispase	D4693-1G	Sigma-Aldrich (St. Louis, MO, USA)
N-hydroxysuccinimide	NHS	13062	Sigma-Aldrich (St. Louis, MO, USA)
N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride	EDC	3450	
		Cell culture medium and component	
Essential 8 medium	Essential 8	A1517001	Thermo Fisher Scientific (Waltham, MA, USA)
Essential 6 medium	Essential 6	A1516401	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
DMEM/F12 medium	DMEM/F12 medium	11330-057	Thermo Fisher Scientific (Waltham, MA, USA)
RPMI 1640	RPMI 1640	11875093	Thermo Fisher Scientific (Waltham, MA, USA)
Alkaline Phosphatase Assay Kit	SensoLyte® pNPP	AS-72146	AnaSpec, Inc. (Fremont, CA, USA)
DAPI	DAPI	D9542	Sigma-Aldrich (St. Louis, MO, USA)
Hoechst 33342	Hoechst	PA-3014	Lonza (Basel, Switzerland)
B-27™ Supplement, minus insulin	B27-	A1895601	Thermo Fisher Scientific (Waltham, MA, USA)
B-27™ Supplement	B27	17504044	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
CHIR99021	CHIR99021	SML1046	Sigma-Aldrich (St. Louis, MO, USA)
IWR-1	IWR-1	I0161	Sigma-Aldrich (St. Louis, MO, USA)
		Antibodies	
Anti-Oct3/4 antibody	Anti-Oct3/4 antibody	sc-5279	Santa Cruz Biotechnology (Dallas, TX, USA)
Anti-Sox2 antibody	Anti-Sox2 antibody	AB5603	Merck KGaA (Darmstadt, Germany)
Anti-SSEA-4 antibody	Anti-SSEA-4 antibody	ab16287	Abcam (Cambridge, MA, USA)
Anti-Nanog antibody	Anti-Nanog antibody	MA1-017	Thermo Fisher Scientific (Waltham, MA, USA)
Anti-α-actinin antibody	Anti-α-actinin antibody	A7811	Sigma-Aldrich (St. Louis, MO, USA)
Anti-MLC2v antibody	Anti-MLC2v antibody	ab92721	Abcam (Milton, Cambridge, UK)
Anti-MLC2a antibody	Anti-MLC2a antibody	ab50967	Abcam (Milton, Cambridge, UK)
Anti-NKX2.5 antibody	Anti-NKX2,5 antibody	ab97355	Abcam (Milton, Cambridge, UK)
Anti-cTnT antibody	Anti-cTnT antibody	MA5-12960	Thermo Fisher Scientific (Waltham, MA, USA)
Mouse IgG1 Isotype antibody	Isotype-control	MA5-14453	Thermo Fisher Scientific (Waltham, MA, USA)
Alexa Fluor 488 goat anti-mouse IgG	Alexa Fluor 488 goat anti-mouse IgG	A-11001	Thermo Fisher Scientific (Waltham, MA, USA)
Alexa Fluor 488 goat anti-rabbit IgG	Alexa Fluor 488 goat anti-rabbit IgG	A-11008	Thermo Fisher Scientific (Waltham, MA, USA)
Alexa Fluor 555 goat anti-mouse IgG	Alexa Fluor 555 goat anti-mouse IgG	A-21424	Thermo Fisher Scientific (Waltham, MA, USA)
Alexa Fluor 555 goat anti-rabbit IgG	Alexa Fluor 555 goat anti-rabbit IgG	ab150078	Abcam (Milton, Cambridge, UK)
		Mice	
NOD.CB17-Prkdcscid/Jnarl	NOD-SCID mice	NOD.CB17-Prkdcscid/Jnarl	National Laboratory Animal Center (Taipei, Taiwan)



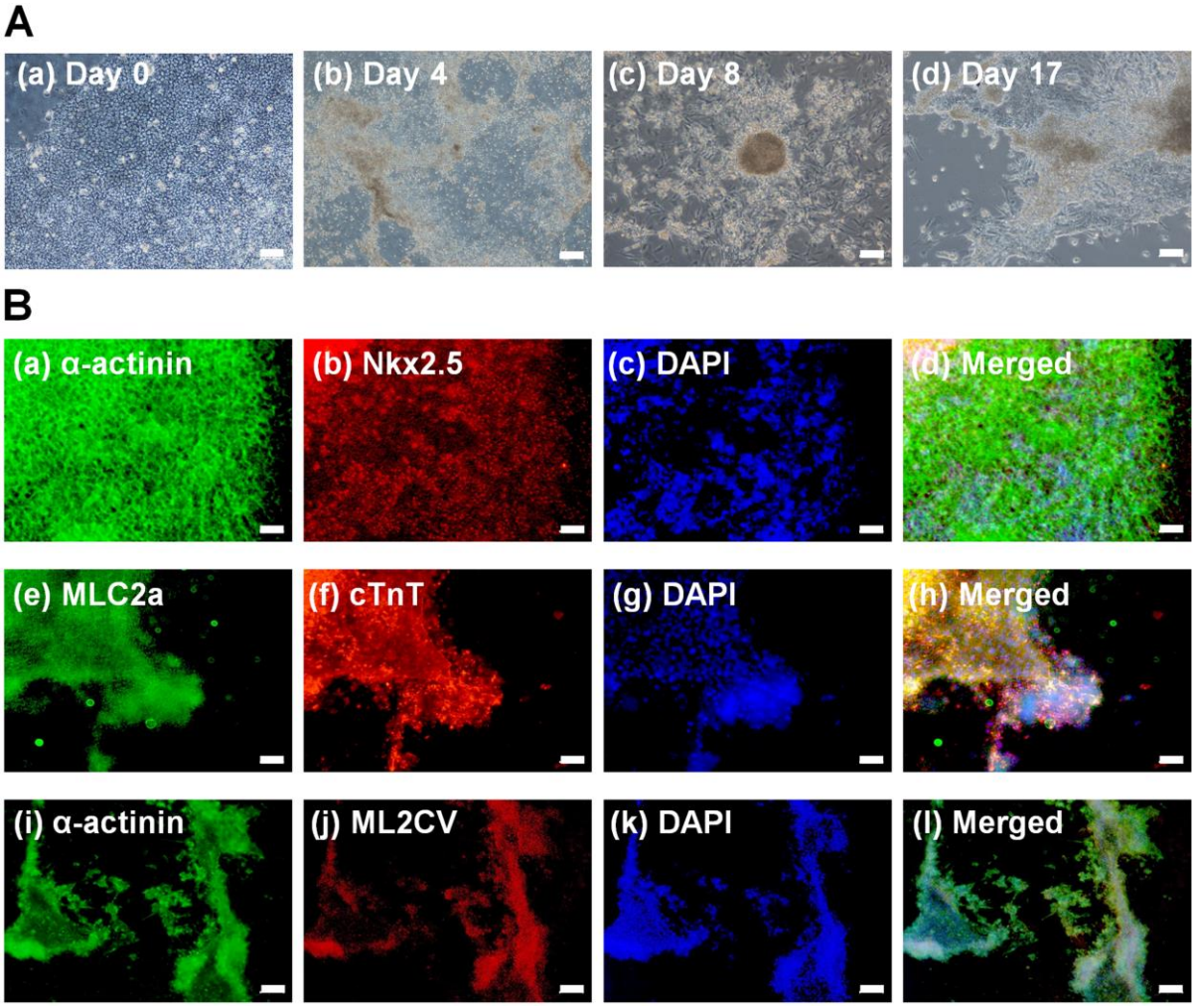
Supplementary Fig. 1. hES (H9) cell culture on peptide-grafted PV hydrogels. (A) Morphologies of hES cells on Matrigel-coated TPS dishes (a), VN1GK-PV hydrogels (b), VN2CK-PV hydrogels (c), LA1G-PV hydrogels (d), LA1GK-PV hydrogels (e), LA2CK-PV hydrogels (f), LB1-PV hydrogels (g), LB1G-PV hydrogels (h), LB1GK-PV hydrogels (i), and LB2CK-PV hydrogels (j). The scale bar indicates 500 μm.



Supplementary Fig. 2. Pluripotent protein expression of hPS cells on LB1GK-PV hydrogels after long-term (passage 10) culture on LB1GK-PV hydrogels in xeno-free culture conditions. (A) Pluripotency protein expression of Oct3/4 (i, green), Nanog (ii, red), Sox2 (v, green), and SSEA-4 (vi, red) in hES (H9) cells assayed by immunohistochemical staining, with nuclear staining by Hoechst 33342 (blue, iii, vii). The pictures (iv) and (viii) were created by merging (i) – (iii) and (v) – (vii), respectively. Scale bar indicates 100 μm. (B) Pluripotency protein expression of Oct3/4 (i, green), Nanog (ii, red), Sox2 (v, green), and SSEA-4 (vi, red) in hiPS (HPS0077) cells assayed by immunohistochemical staining, with nuclear staining by Hoechst 33342 (blue, iii, vii). The pictures (iv) and (viii) were created by merging (i) – (iii) and (v) – (vii), respectively. Scale bar shows 100 μm.



Supplementary Fig. 3. Evaluation of the differentiation ability of hPS cells *in vitro* after long-term (passage 10) culture on LB1GK-PV hydrogels in xeno-free culture conditions. (A, B) The differentiation of hES (H9) cells (A) and hiPS (HPS0077) cells (B) *in vitro*. (i) Morphology of EB cells differentiated from hPS cells after cultivation of hPS cells on LB1GK-PV hydrogels in xeno-free culture conditions for 10 passages. Expression of a mesodermal marker protein (ii, α-SMA, green), an ectodermal marker protein (v, GFAP, red) and an endodermal marker protein (vi, AFP, green) from EB cells analyzed by immunohistochemical staining with nuclear staining from Hoechst 33342 (iii, vii, blue) after the cultivation of hPS cells on LB1GK-PV hydrogels in xeno-free culture conditions for 10 passages. The photos (iv) and (viii) were created by merging (ii) – (iii) and (v) – (vii), respectively. Scale bar indicates 200 μm for (i) and 100 μm for (ii) – (viii).



Supplementary Fig. 4. Cardiomyocyte differentiation of hES (H9) cells after cultivation of hES cells on LB2CK-PV hydrogels in xeno-free culture conditions for 10 passages. (A) The sequential morphological observation during hES cell differentiation on LB2CK-PV hydrogels at day 0, 4, 8, and 17. Scale bar shows 100 μm. (B) Immunohistochemical staining assay of hES cell-derived cardiomyocytes on LB2CK-PV hydrogels. Expression of α-actinin (a and i, green), NKX2.5 (b, red), MLC2a (e, green), cTnT (f, red) and ML2Cv (j, red) on hES cell-derived cardiomyocytes assayed by an immunohistochemical staining, which were induced differentiation on LB2CK-PV hydrogels on day 21. DAPI (c, g and k) was used for nuclei staining. The photos (d), (h) and (l) were created by merging (a) – (c), (e) – (g), and (i) – (k), respectively. The scale bar shows 100 μm.