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Designed peptide-grafted hydrogels for human pluripotent stem cell culture and differentiation

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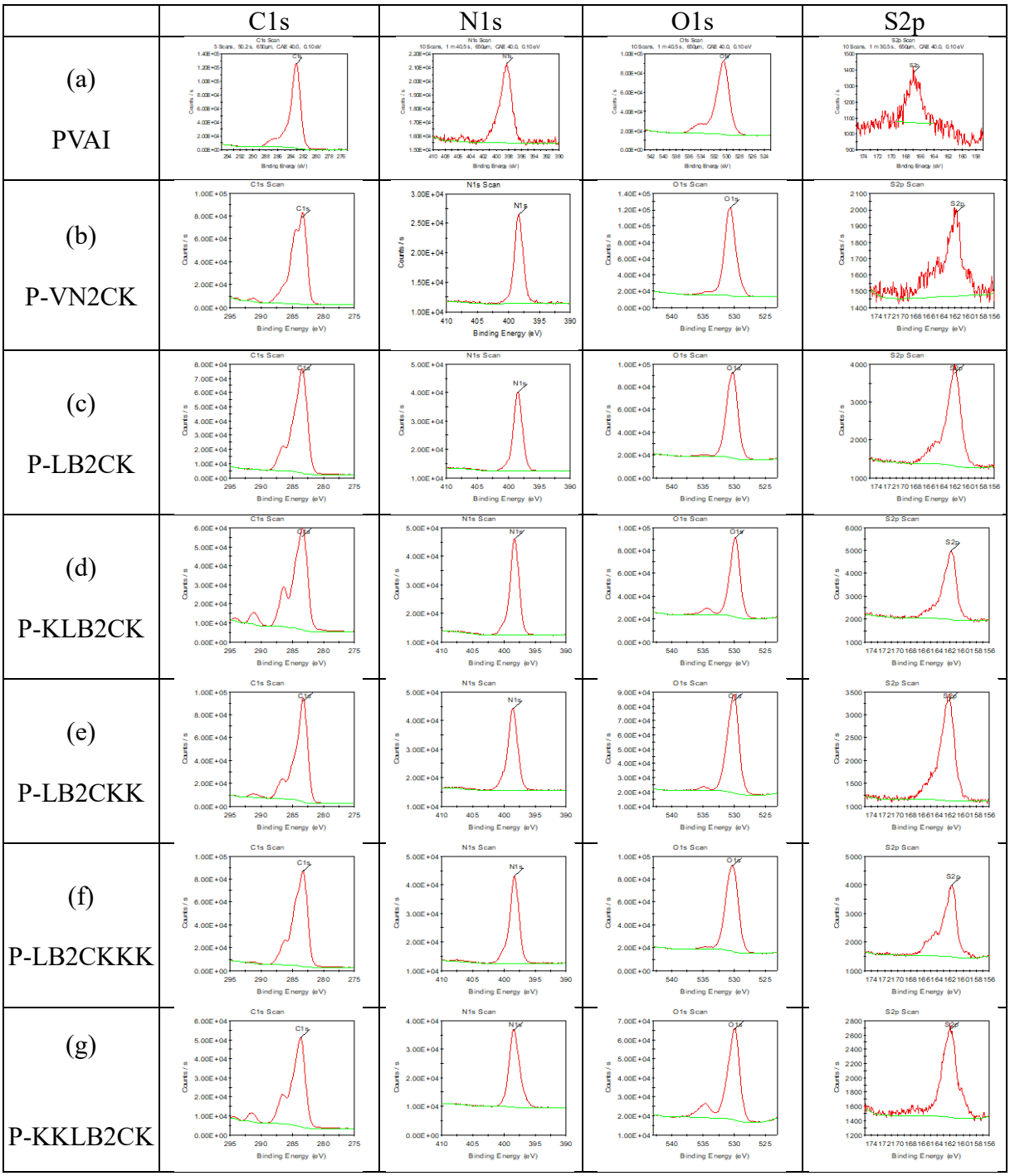
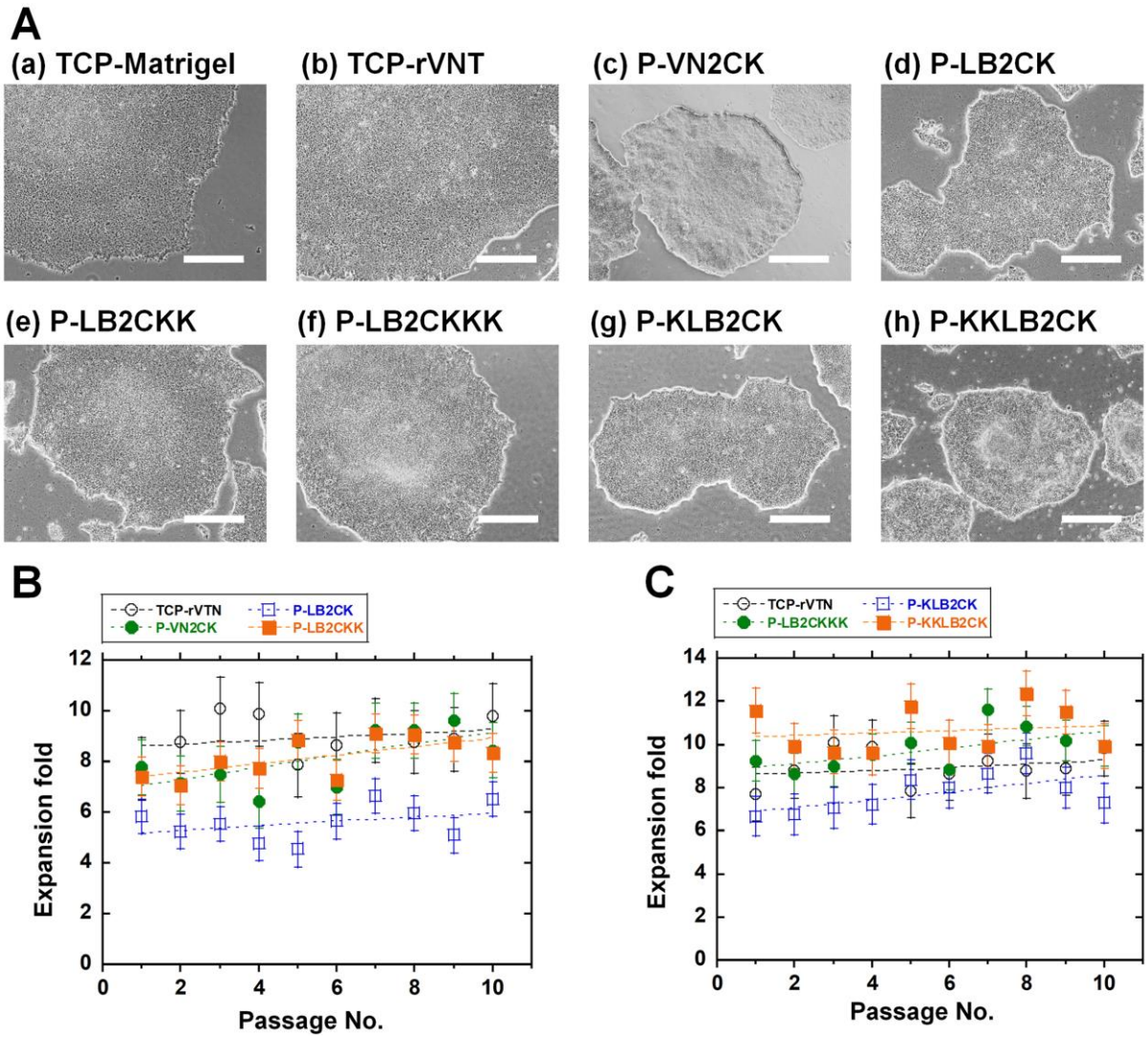
Supplementary Information

Supplementary Table 1 Materials used in this study.

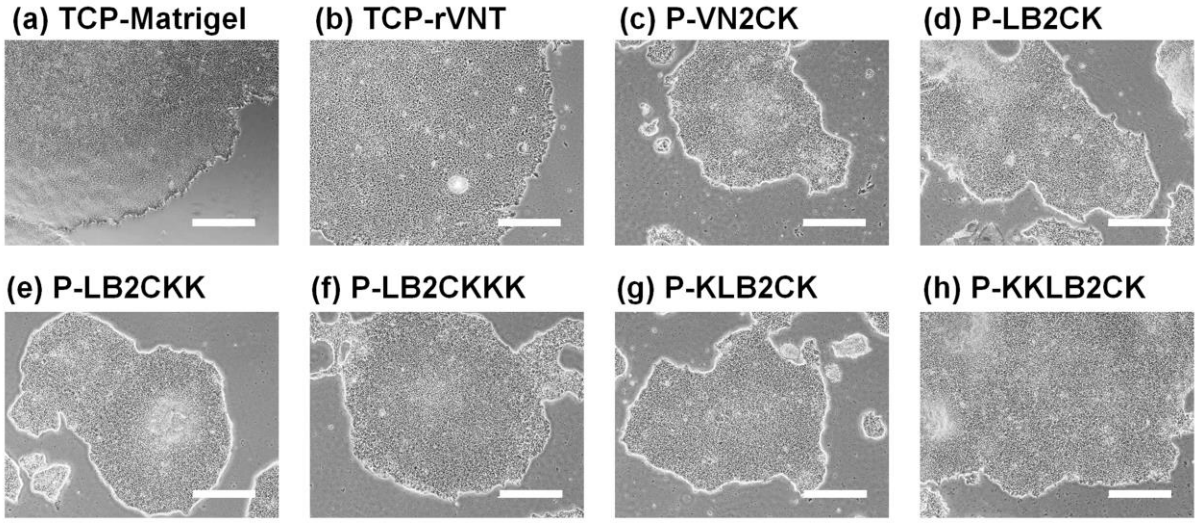
Materials	Abbreviation	Catalog No.	Company
		ECM	
Matrigel	MAT	356230	Corning (Corning, NY, USA)
Recombinant vitronectin	rVTN	A14700	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
		Cell culture dishes	
6-well polystyrene plate	TCP	#353046	Corning (Corning, NY, USA)
		Chemicals	
N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride	EDC	E7750	Sigma-Aldrich (St. Louis, MO, USA)
N-Hydroxysuccinimide	NHS	56480	Sigma-Aldrich (St. Louis, MO, USA)
Dispase II	Dispase	D4693-1G	Sigma-Aldrich (St. Louis, MO, USA)
Trypsin-EDTA (0.25%)	Trypsin-EDTA	25200072	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
Bone morphogenic protein 4	BMP4	H4916	Sigma-Aldrich (St. Louis, MO, USA)
3-(6-Methyl-2-pyridinyl)-N-phenyl-4-(4-quinolinyl)-1H-pyrazole-1-carbothioamide	A83-01	SML0788	Sigma-Aldrich (St. Louis, MO, USA)
β-glycerophosphate	β-GP	G9422-10G	Sigma-Aldrich (St. Louis, MO, USA)
L-ascorbic acid-2-phosphate	L-AA	A8960-5G	Sigma-Aldrich (St. Louis, MO, USA)
Dexamethasone	DX	D4902-500G	Sigma-Aldrich (St. Louis, MO, USA)
		Cell culture medium and component	
Essential 8 medium	E8	A1517001	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
Essential 6 medium	E6	A1516401	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
DMEM/F12 medium	DMEM/F12	11330-057	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
α-MEM	α-MEM	12000022	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
DMEM	DMEM	D5648	Sigma-Aldrich (St. Louis, MO, USA)
Fetal bovine serum	FBS	PSRPS-FB2	Biological Industries (Kibbutz Beit-Haemek, Israel)
		Antibodies	
Mouse Anti-GFAP antibody	Anti-GFAP antibody	MA5-15086	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
Mouse Anti-AFP antibody	Anti-AFP antibody	PA5-21004	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
Rabbit Anti-SMA antibody	Anti-SMA antibody	PA5-19465	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
Alexa Fluor® 488 Goat Anti-Rabbit IgG (H+L) Antibody	488 Anti-Rabbit IgG Antibody	A11008	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
Alexa Fluor® 594 Donkey Anti-Mouse IgG (H+L) Antibody	594 Donkey Anti-Mouse IgG (H+L) Antibody	A21203	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
FITC Mouse Anti-Human CD34	Anti-CD34 antibody	555821	BD Pharmingen (Franklin Lakes, NJ, USA)
FITC Mouse IgG1	Anti-CD34 isotype antibody	555748	BD Pharmingen (Franklin Lakes, NJ, USA)
FITC Mouse Anti-Human CD44	Anti-CD44 antibody	555478	BD Pharmingen (Franklin Lakes, NJ, USA)
FITC Mouse IgG2b	Anti-CD44 isotype antibody	555742	BD Pharmingen (Franklin Lakes, NJ, USA)
PE Mouse Anti-Human CD73	Anti-CD73 antibody	550257	BD Pharmingen (Franklin Lakes, NJ, USA)
PE Mouse Anti-Human CD105	Anti-CD105 antibody	A07414	Beckman Coulter (Brea, CA, USA)
PE Mouse IgG3 RPE	Anti-CD105 isotype antibody	0105-09	SoutherBiotech (Birmingham, AL, USA)



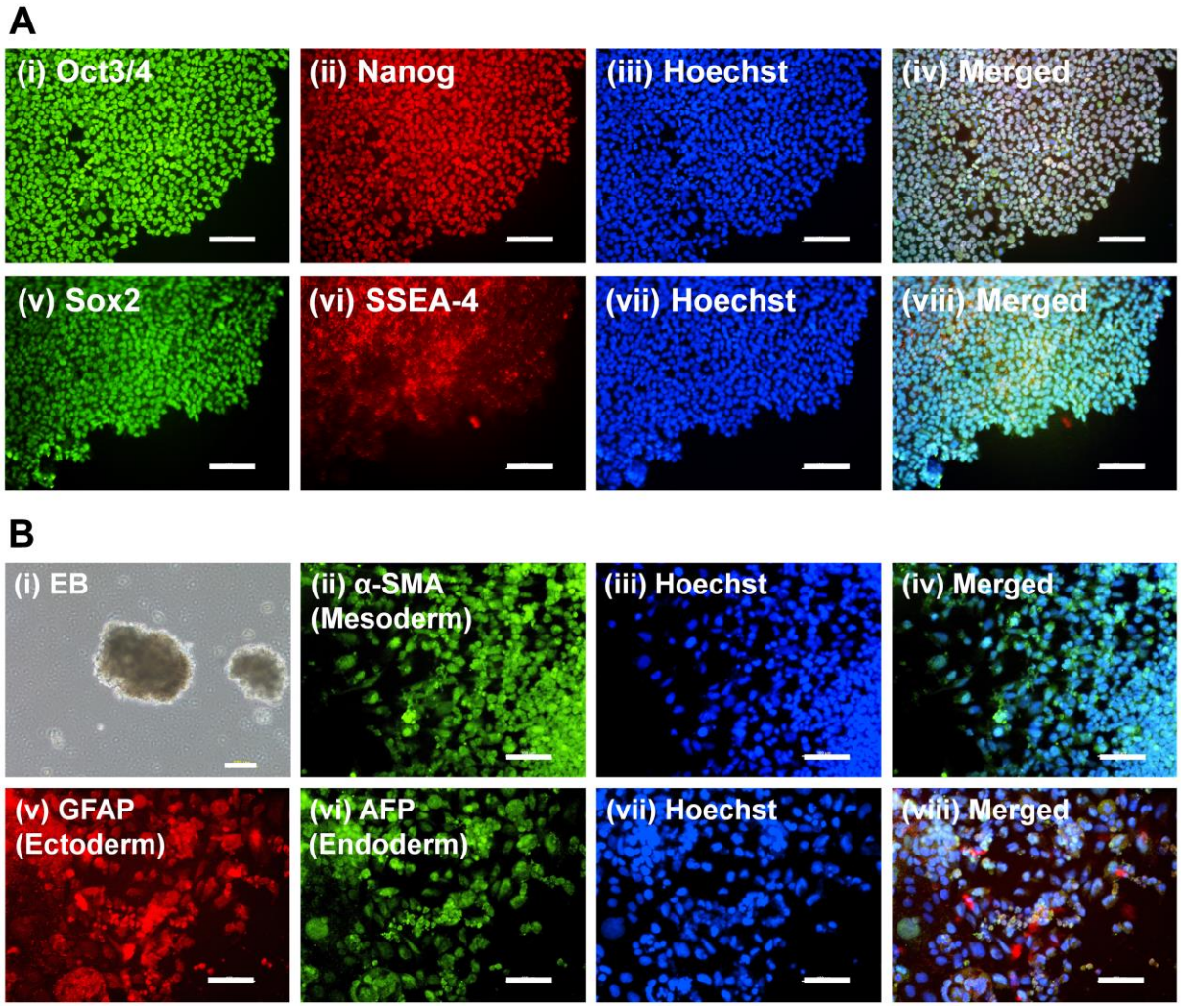
Supplementary Fig. 1 Surface analysis of PVAI hydrogels conjugated with designed peptides by XPS. High-resolution spectra of the C 1s, N 1s, O 1s and S 2p peaks of the surfaces of the PVAI (a), P-VN2CK (b), P-LB2CK (c), P-KLB2CK (d), P-LB2CKK (e), P-LB2CKKK (f) and P-KKLB2CK (g) hydrogels.



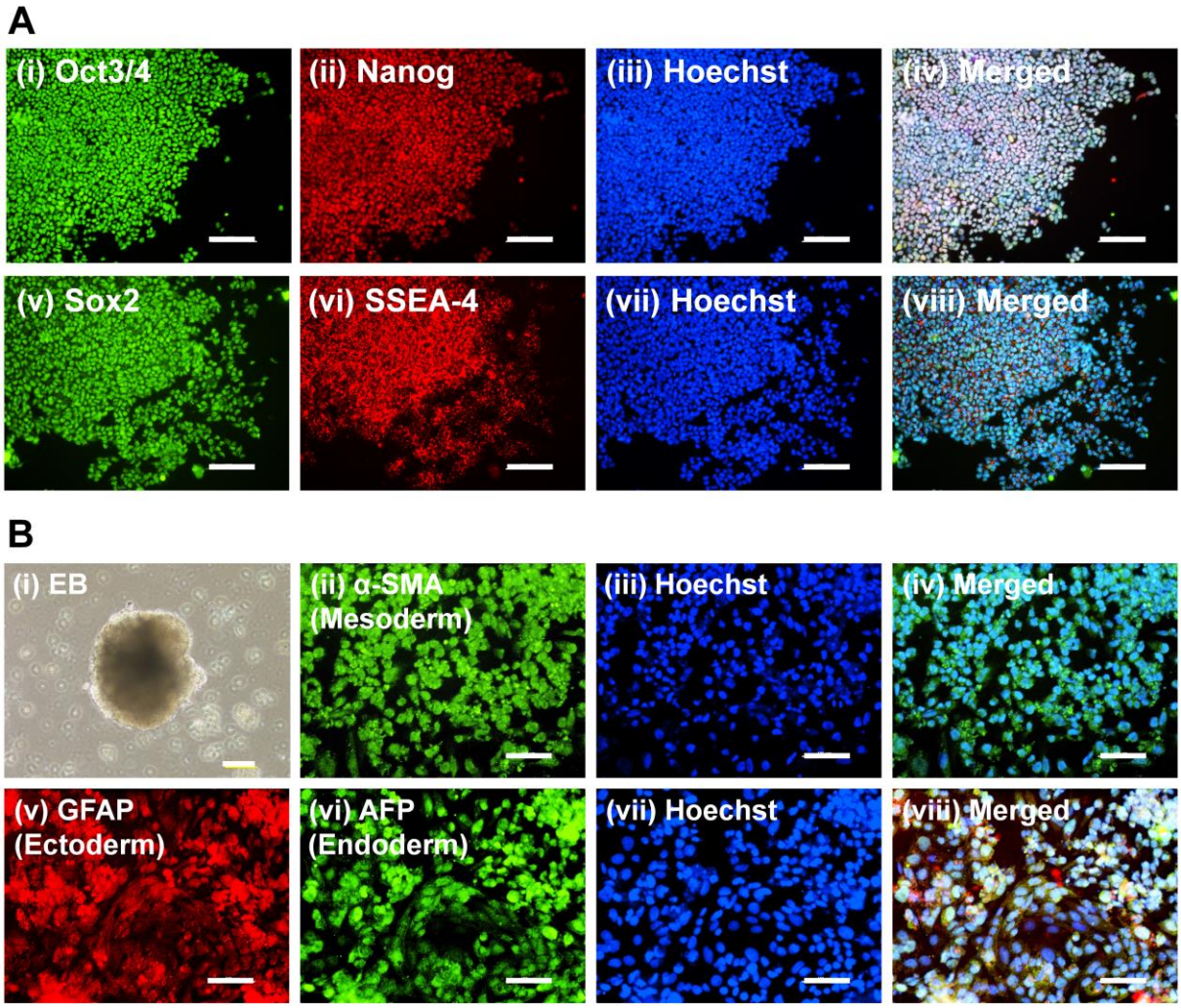
Supplementary Fig. 2. hiPSC (HPS0077) cultivation on PVAI hydrogels conjugated with designed peptides under xeno-free culture conditions. (A) Morphologies of hiPSCs cells on Matrigel-coated TCP (TCP-Matrigel) plates (a), rVNT-coated TCP (TCP-rVNT) plates (b), P-VN2CK hydrogels (c), P-LB2CK hydrogels (d), P-LB2CKK hydrogels (e), P-LB2CKKK hydrogels (f), P-KLB2CK hydrogels (g), and P-KKLB2CK hydrogels (h) at passage 10. The scale bar is 500 μm. (B) Passage number dependence of the expansion fold of hiPSCs on TCP-rVNT plates and PVAI hydrogels conjugated with designed peptides (P-VN2CK, P-LB2CK and P-LB2CKK). (C) Passage number dependence of the expansion fold of hiPSCs on TCP-rVNT plates and PVAI hydrogels conjugated with designed peptides (P-LB2CKKK, P-KLB2CK and P-KKLB2CK).



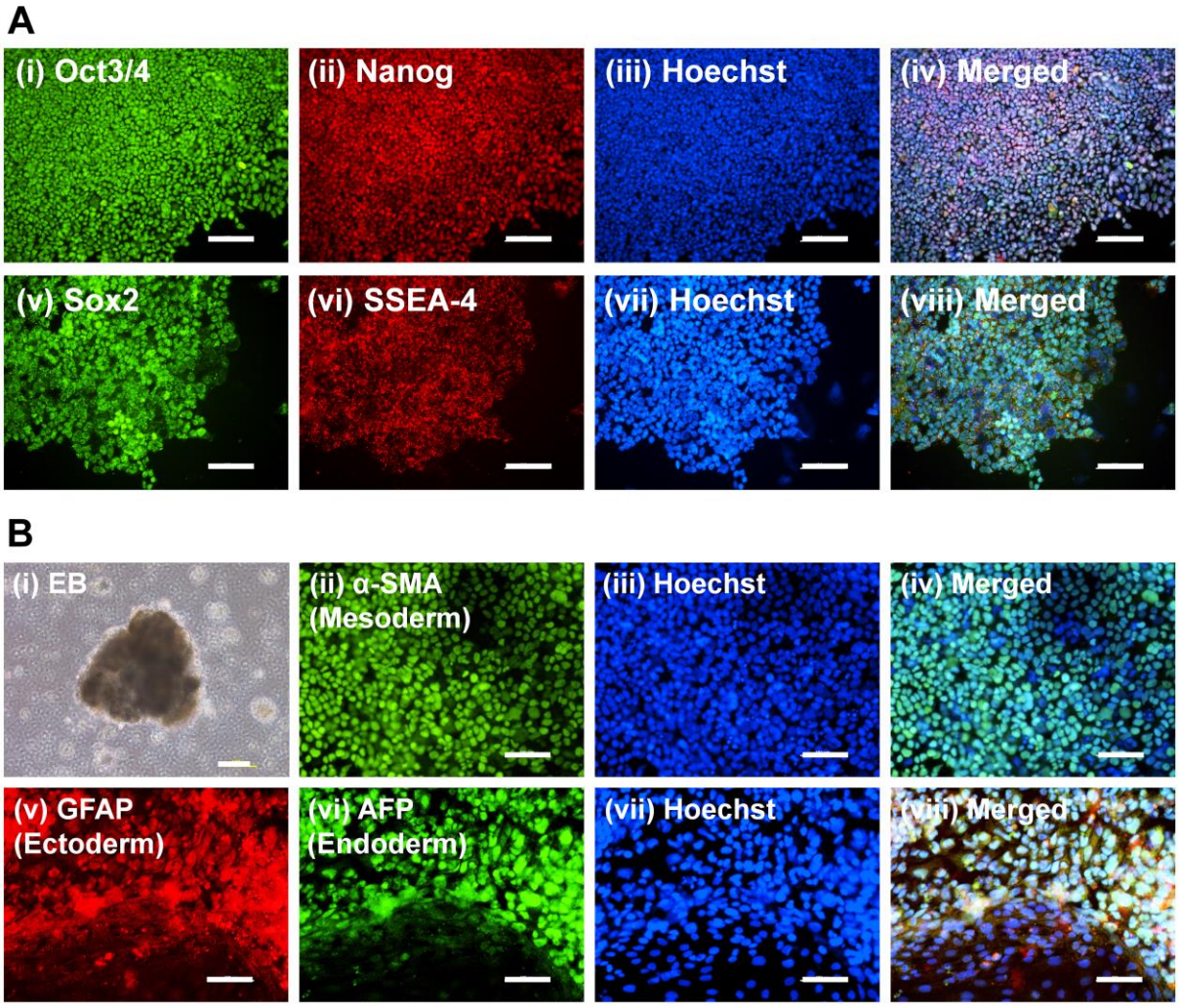
Supplementary Fig. 3. hESC (H9) cultivation on PVAI hydrogels conjugated with designed peptides under xeno-free culture conditions. Morphologies of hESCs cells on Matrigel-coated TCP (TCP-Matrigel) plates (a), rVNT-coated TCP (TCP-rVNT) plates (b), P-VN2CK hydrogels (c), P-LB2CK hydrogels (d), P-LB2CKK hydrogels (e), P-LB2CKKK hydrogels (f), P-KLB2CK hydrogels (g), and P-KKLB2CK hydrogels (h) at passage 10. The scale bar is 500 μm.



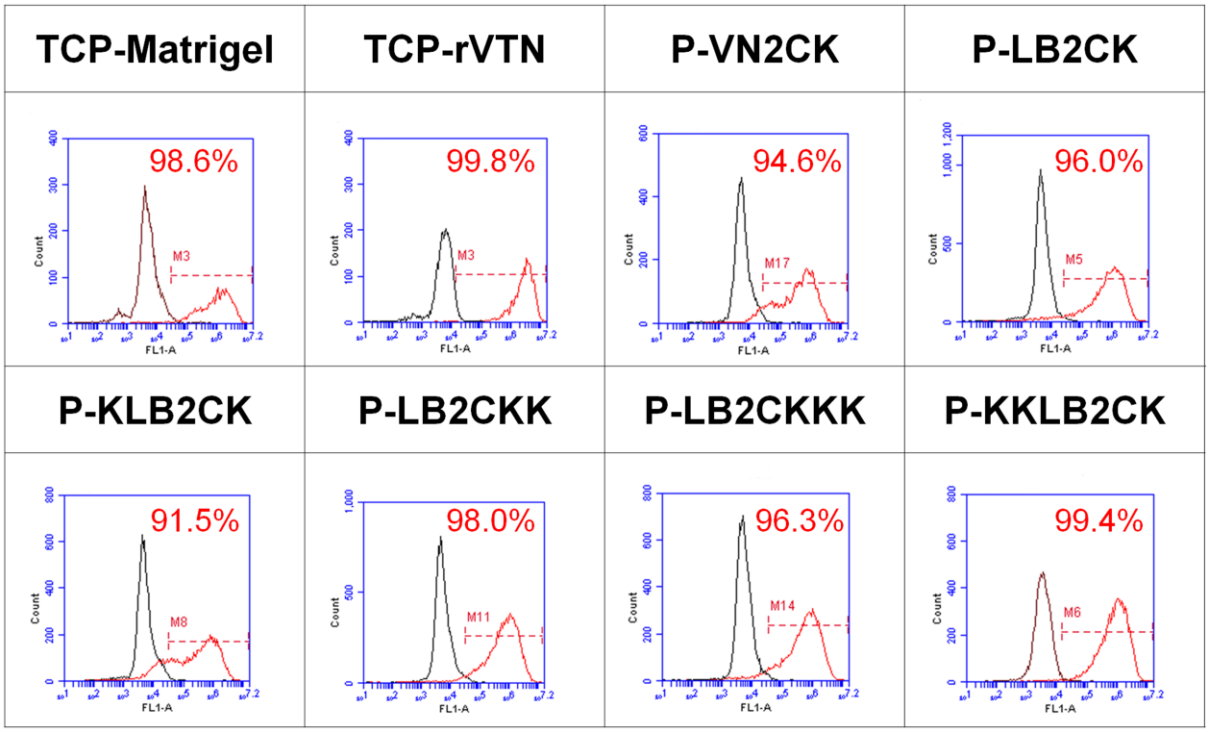
Supplementary Fig. 4. Pluripotency and *in vitro* differentiation ability of hiPSCs (HPS0077) after long-term (passage ten) cultivation on P-LB2CKKK hydrogels using xeno-free cultivation protocols. (A) Pluripotency protein expression of Oct3/4 (i, green), Nanog (ii, red), Sox2 (v, green), and SSEA-4 (vi, red) in hiPSCs (HPS0077) analyzed using immunostaining with nuclear staining by Hoechst 33342 (blue, iii, vii) after hiPSC culture on P-LB2CKKK hydrogels using xeno-free cultivation protocols for ten passages. The images (iv) and (viii) were generated by merging (i)–(iii) and (v)–(vii), respectively. The scale bar is 100 μm. (B) (i) Morphology of EB cells differentiated from hiPSCs (HPS0077) after hiPSC culture on P-LB2CKKK hydrogels using xeno-free cultivation protocols for ten 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 evaluated using immunostaining with nuclear staining from Hoechst 33342 (iii, vii, blue) after hiPSC culture on P-LB2CKKK hydrogels using xeno-free cultivation protocols for ten passages. The photos (iv) and (viii) were generated by merging (ii)–(iii) and (v)–(vii), respectively. The scale bar is 100 μm.



Supplementary Fig. 5. Pluripotency and *in vitro* differentiation ability of hiPSCs (H-M5) after long-term (passage ten) cultivation on P-KKLB2CK hydrogels using xeno-free cultivation protocols. (A) Pluripotency protein expression of Oct3/4 (i, green), Nanog (ii, red), Sox2 (v, green), and SSEA-4 (vi, red) in hiPSCs (H-M5) analyzed using immunostaining with nuclear staining by Hoechst 33342 (blue, iii, vii) after hiPSC culture on P-KKLB2CK hydrogels using xeno-free cultivation protocols for ten passages. The images (iv) and (viii) were generated by merging (i)–(iii) and (v)–(vii), respectively. The scale bar is 100 μm. (B) (i) Morphology of EB cells differentiated from hiPSCs (H-M5) after hiPSC culture on P-KKLB2CK hydrogels using xeno-free cultivation protocols for ten 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 evaluated using immunostaining with nuclear staining from Hoechst 33342 (iii, vii, blue) after hiPSC culture on P-KKLB2CK hydrogels using xeno-free cultivation protocols for ten passages. The photos (iv) and (viii) were generated by merging (ii)–(iii) and (v)–(vii), respectively. The scale bar is 100 μm.



Supplementary Fig. 6. Pluripotency and *in vitro* differentiation ability of hiPSCs (H-M5) after long-term (passage ten) cultivation on P-LB2CKKK hydrogels using xeno-free cultivation protocols. (A) Pluripotency protein expression of Oct3/4 (i, green), Nanog (ii, red), Sox2 (v, green), and SSEA-4 (vi, red) in hiPSCs (H-M5) analyzed using immunostaining with nuclear staining by Hoechst 33342 (blue, iii, vii) after hiPSC culture on P-LB2CKKK hydrogels using xeno-free cultivation protocols for ten passages. The images (iv) and (viii) were generated by merging (i)–(iii) and (v)–(vii), respectively. The scale bar is 100 μm. (B) (i) Morphology of EB cells differentiated from hiPSCs (H-M5) after hiPSC culture on P-LB2CKKK hydrogels using xeno-free cultivation protocols for ten 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 evaluated using immunostaining with nuclear staining from Hoechst 33342 (iii, vii, blue) after hiPSC culture on P-LB2CKKK hydrogels using xeno-free cultivation protocols for ten passages. The photos (iv) and (viii) were generated by merging (ii)–(iii) and (v)–(vii), respectively. The scale bar is 100 μm.



Supplementary Fig. 7. Pluripotent marker expression of SSEA-4 by hiPSCs (HPS0077) after culture on TCP-Matrigel dishes, TCP-rVTN dishes and each PVAI hydrogel conjugated with designed peptides for 10 passages under xeno-free cultivation conditions, which was evaluated using flow cytometry.