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Laminin-511 and recombinant vitronectin supplementation enables human pluripotent stem cell culture and differentiation on conventional tissue culture polystyrene surface in xeno-free conditions

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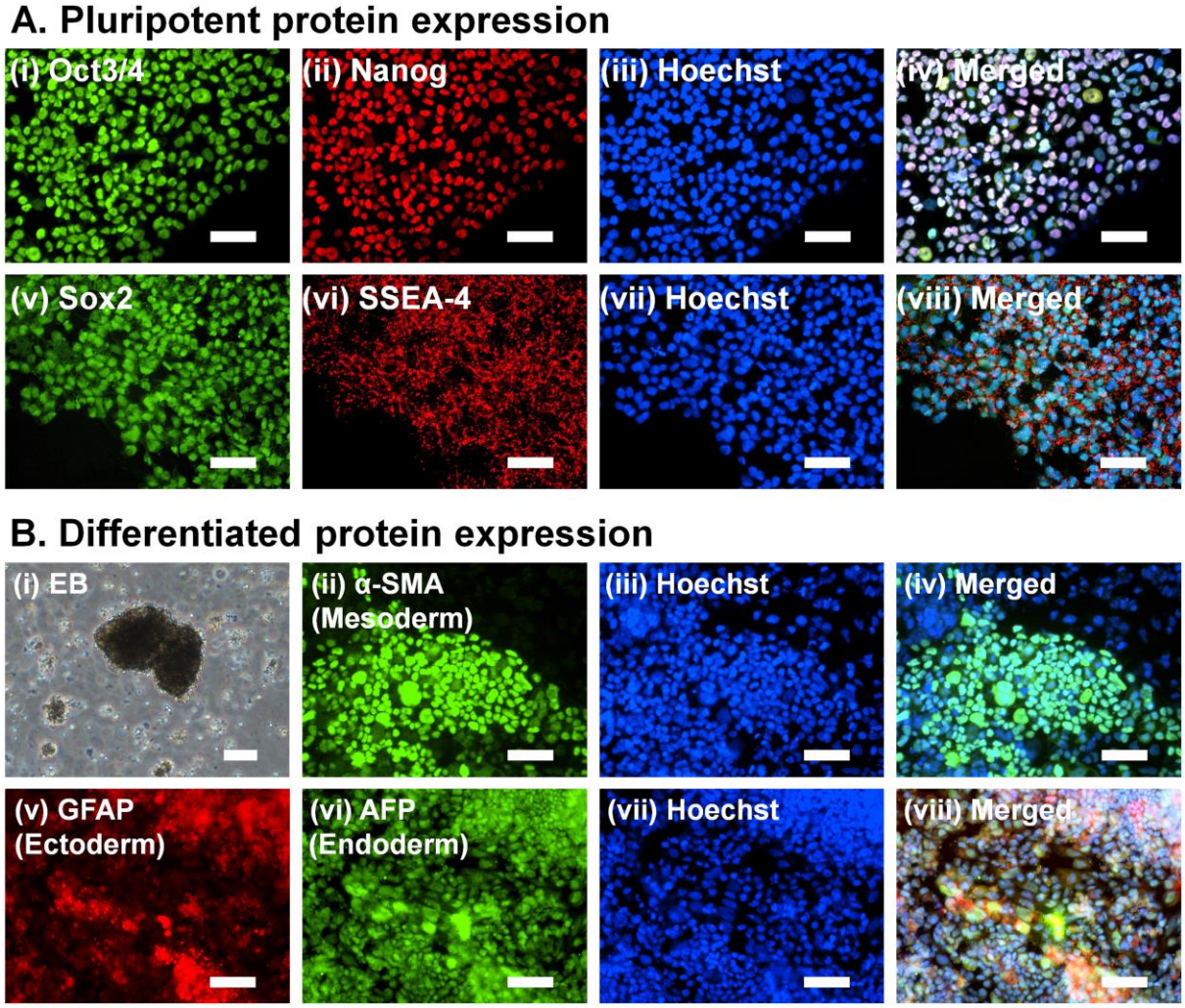
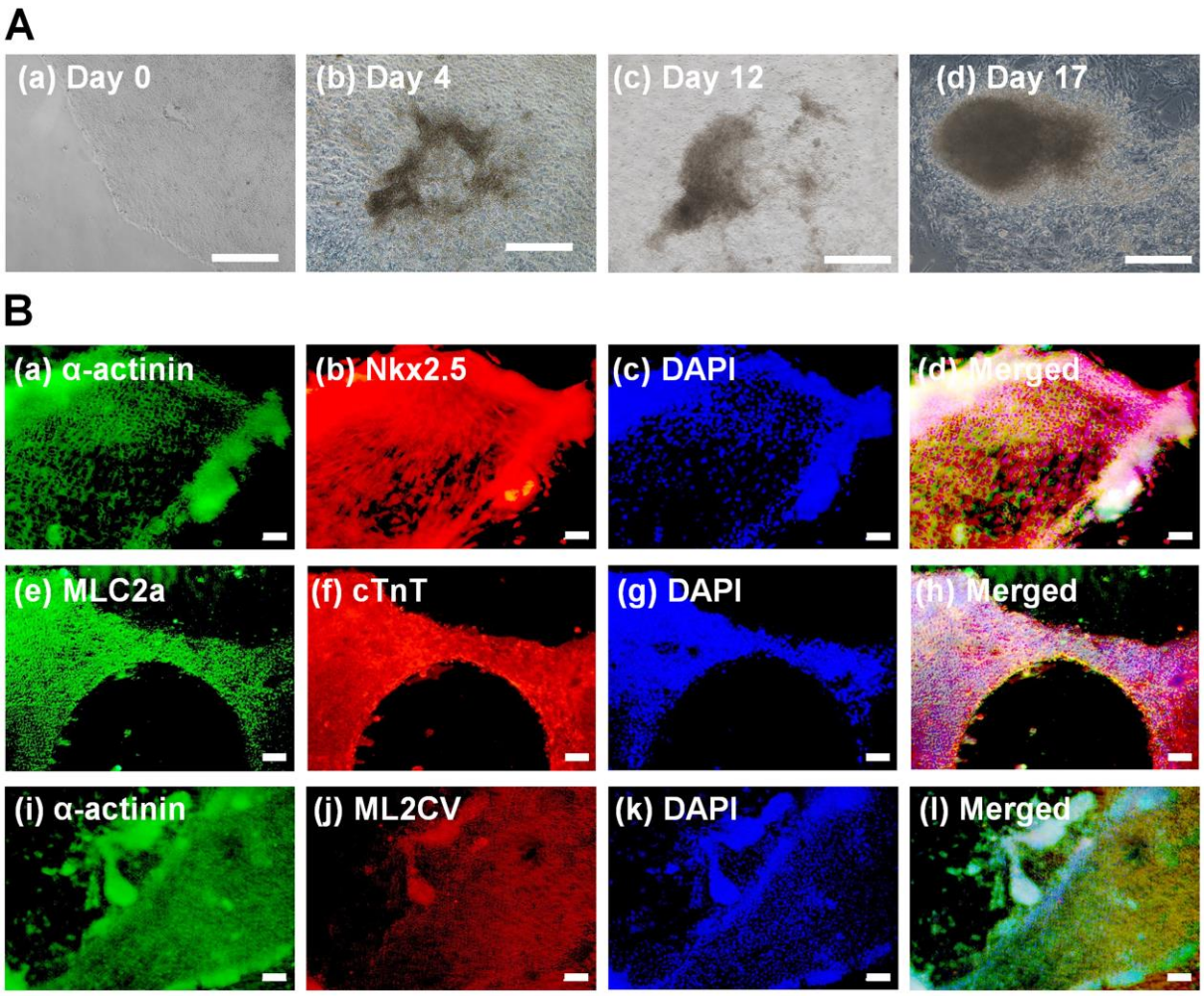
Supplementary Information

Supplementary Table 1 Materials used in this study.

Materials	Abbreviation	Catalog No.	Company
		ECM	
Matrigel	Matrigel	#356230	Corning (Corning, NY, USA)
Recombinant vitronectin	rVT	A14700	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
Laminin 511	L-511	892011	Nippi Inc. (Tokyo, Japan)
Laminin 521	L-521	BLA-LN521-02	Veritas (Tokyo, Japan)
		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	Sigma-Aldrich (St. Louis, MO, USA)
		Cell culture medium and component	
Essential 8 medium	Essential 8	A1517001	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
Essential 6 medium	Essential 6	A1516401	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
DMEM/F12 medium	DMEM/F12	11330-057	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
RPMI 1640	RPMI 1640	11875093	Thermo Fisher Scientific Inc. (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 Inc. (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 Inc. (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 Inc. (Waltham, MA, USA)
Mouse IgG1 Isotype antibody	Isotype-control	MA5-14453	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
Alexa Fluor 488 goat anti-mouse IgG	Alexa Fluor 488 goat anti-mouse IgG	A-11001	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
Alexa Fluor 488 goat anti-rabbit IgG	Alexa Fluor 488 goat anti-rabbit IgG	A-11008	Thermo Fisher Scientific Inc. (Waltham, MA, USA)
Alexa Fluor 555 goat anti-mouse IgG	Alexa Fluor 555 goat anti-mouse IgG	A-21424	Thermo Fisher Scientific Inc. (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 Pluripotent protein and differentiation protein expression of hiPSCs (H-M5) after long-term cultivation for ten passages on untreated TCP dishes in the supplemented method using Mix-0.2 conditionS in xeno-free culture condition. (A) Pluripotency protein expression of Oct3/4 (i, green), Nanog (ii, red), Sox2 (v, green), and SSEA-4 (vi, red) in hiPSCs (H-M5) evaluated by immunostaining method, with nuclear staining by Hoechst 33342 (blue, iii, vii). The photos (iv) and (viii) were merged from the photos of (i) – (iii) and (v) – (vii), respectively. Scale bar indicates 100 μm. (B) Evaluation of the differentiation ability of hiPSCs (H-M5) *in vitro* after long-term cultivation for ten passages on untreated TCP dishes in the supplemented method using Mix-0.2 condition in xeno-free culture condition. Morphology of EB cells differentiated from hPSCs (i). 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 by the immunostaining method, with nuclear staining from Hoechst 33342 (iii, vii, blue). The photos (iv) and (viii) were merged from the photos of (ii) – (iii) and (v) – (vii), respectively. The scale bar indicates 100 μm.



Supplementary Fig. 2 Cardiac differentiation of hESCs (H9) after hESC culture for ten passages on untreated TCP dishes in the supplemented method using Mix-0.2 condition in xeno-free culture condition. (A) Timeline of the differentiation protocol for hESCs into cardiomyocytes. (B) The sequential morphological observation during cardiac induction of hESCs at day 0, 4, 12, and 17. The scale bar shows 100 μm. (C) Immunostaining evaluation of hESC-derived cardiomyocytes. Expression of α-actinin (a and i, green), NKX2.5 (b, red), MLC2a (e, green), cTnT (f, red) and ML2Cv (j, red) on hESC-derived cardiomyocytes after 21 day of differentiation. DAPI (c, g, and k) was utilized 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. (D) The expression of cardiac marker, cTnT on hESC-induced cardiomyocytes were evaluated using flow cytometry.