Supplementary Information

Xeno-free and feeder-free culture and differentiation of human embryonic stem cells on recombinant vitronectin-grafted hydrogels

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Supplementary Figures and Notes

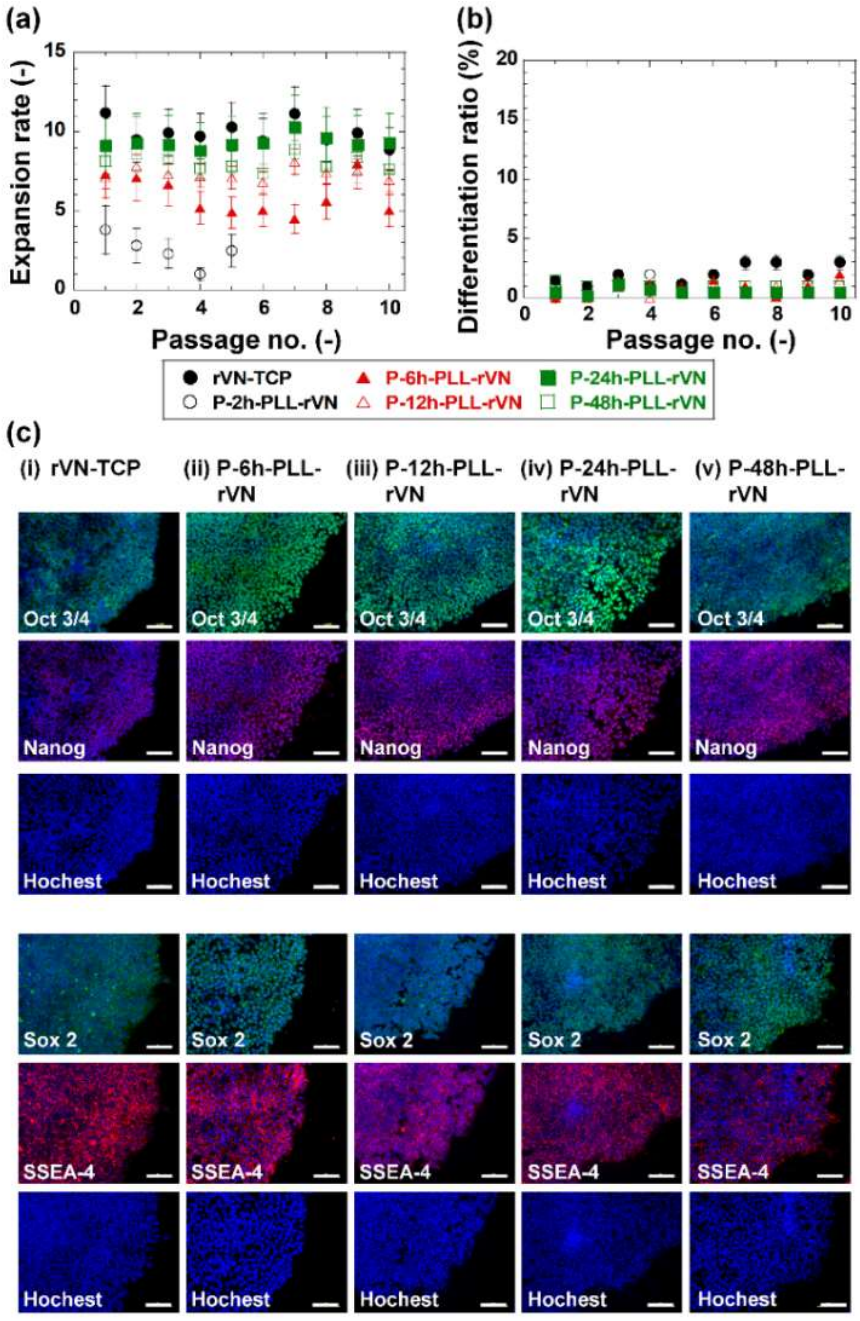

Fig. 1S Long-term culture and characterization of HPS0077 human induced pluripotent stem cells (hiPSCs) on PVI hydrogels grafted with PLL and rVN under feeder-free and xeno-free culture conditions. (a, b) Expansion rate (a) and differentiation ratio (b) of HPS0077 hiPSCs on rVN-TCP dishes prepared with 5 μg/mL rVN coating solution (closed circles), P-2h-PLL-rVN hydrogels (open circles), P-6h-PLL-rVN hydrogels (open squares), and P-24h-PLL-rVN hydrogels (closed squares) for 10 passages. P-*X*h-PLL-rVN hydrogels were prepared with 1 mg/mL PLL solution and 10 μg/mL rVN solution. (c) Expression of pluripotency proteins SSEA-4 (red), Sox2 (green), Nanog (red), and Oct3/4 (green) on HPS0077 hiPSCs evaluated by immunostaining with dual staining with Hoechest33342 for nuclear (blue) after culturing on (i) rVN-TCP dishes prepared with 5 μg/mL rVN coating solution, (ii) P-6h-PLL-rVN hydrogels, (iii) P-12h-PLL-rVN hydrogels, (iv) P-24h-PLL-rVN hydrogels, and (v) P-48h-PLL-rVN hydrogels for 10 passages. P-*X*h-PLL-rVN hydrogels were prepared with 1 mg/mL PLL solution and 10 μg/mL rVN solution. Scale bar indicates 100 μm.

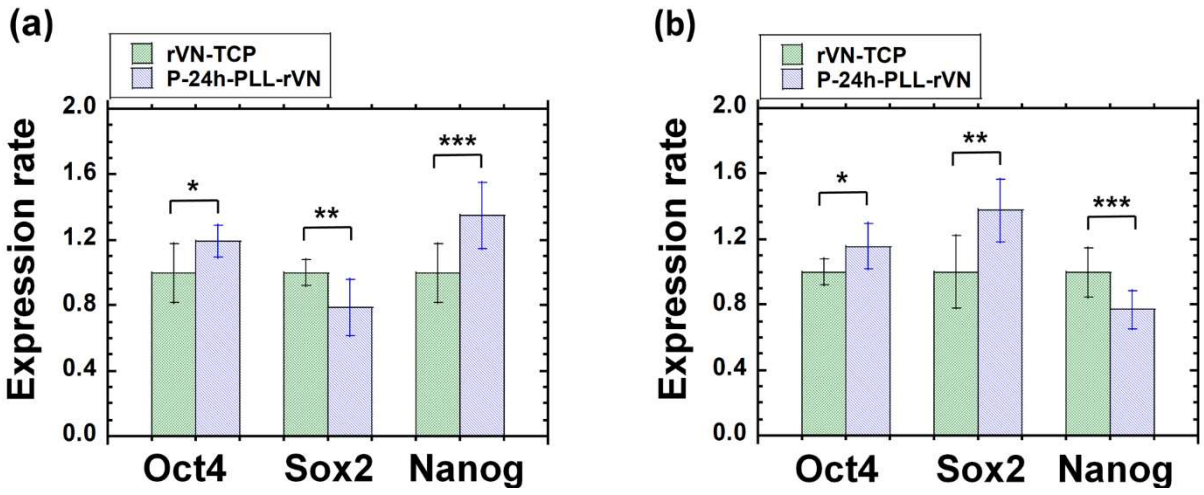

Fig. 2S Relative gene expression levels of *Oct4*, *Sox2*, and *Nanog* as analyzed by qRT-PCR in (a) hESCs and (b) hiPSCs after culturing on P-24h-PLL-rVN hydrogels or rVN-TCP dishes for 10 passages. The gene expression levels were standardized with the levels expressed of hESCs of hiPSCs after culturing on rVN-TCP dishes for 10 passages. *$p > 0.05$; **$p > 0.05$.

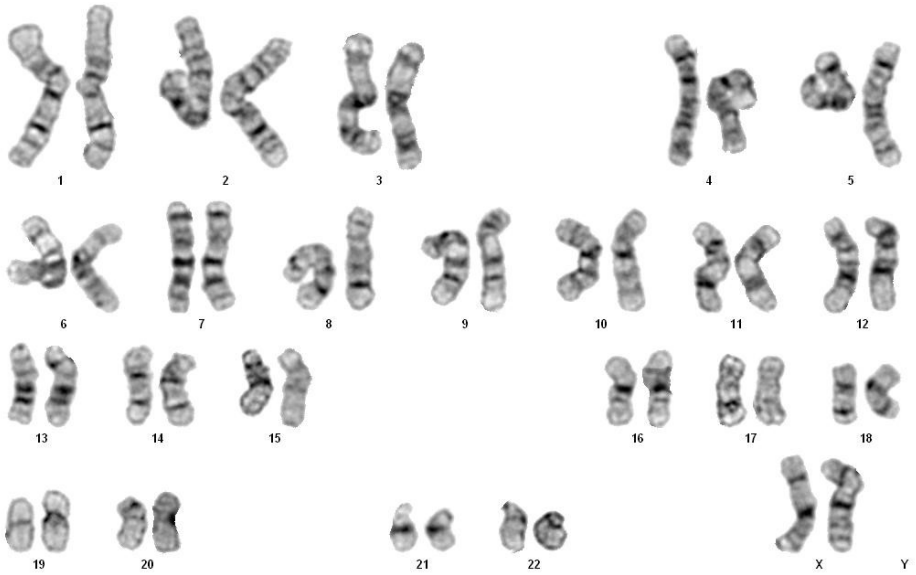

Fig. 3S Karyotyping analysis of hiPSCs after culturing on P-24h-PLL-rVN for 10 passages.

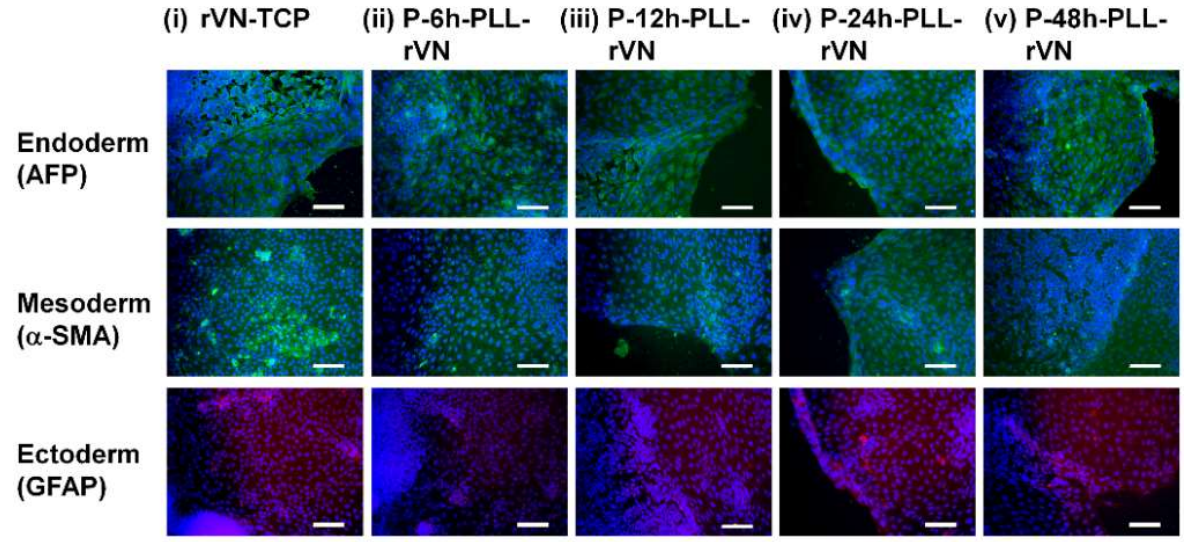

Fig. 4S Characterization of the differentiation ability of HPS0077 hiPSCs in vitro and in vivo after culturing on PVI hydrogels. (b) Expression of an ectoderm protein (GFAP, red), mesoderm protein (α-SMA, green), and endoderm protein (AFP, green) in HPS0077 hiPSCs analyzed by immunostaining with dual staining with Hoechest33342 for nuclear (blue) after culturing on (i) rVN-TCP(5) dishes and (ii) P-6h-PLL-rVN, (iii) P-12h-PLL-rVN, (iv) P-24h-PLL-rVN, and (v) P-48h-PLL-rVN hydrogels for 10 passages. Scale bar indicates 100 μm.

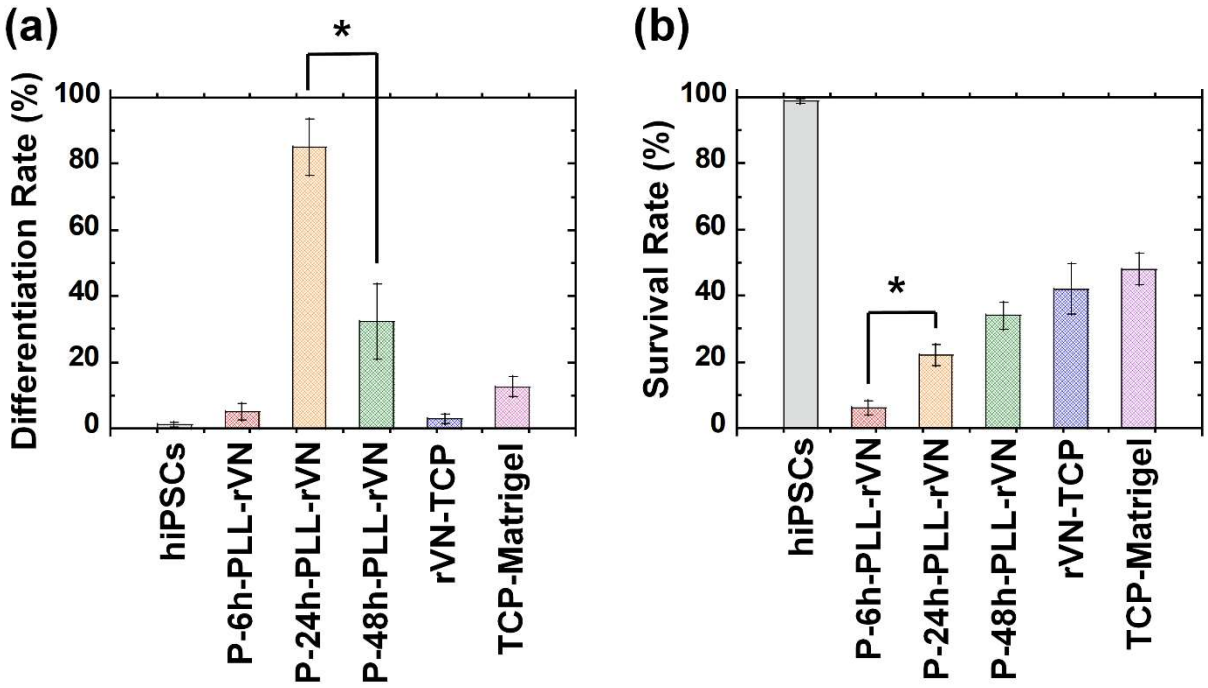

Fig. 5S Characterization of differentiation of HPS0077 hiPSCs into cardiomyocytes during culture on PVI hydrogels grafted with PLL and rVN under feeder-free and xeno-free culture conditions. (a) Differentiation rate of cells from HPS0077 hiPSCs cultivated on P-6h-PLL-rVN hydrogels, P-24h-PLL-rVN hydrogels, P-48h-PLL-rVN hydrogels, rVN-TCP dishes prepared with 5 μg/mL rVN coating solution, and Matrigel-coated dishes after 12 days of induction. Differentiation rate of HPS0077 hiPSCs is also plotted. (b) Survival rate of differentiated HPS0077 hiPSCs cultivated on P-6h-PLL-rVN hydrogels, P-24h-PLL-rVN hydrogels, P-48h-PLL-rVN hydrogels, rVN-TCP dishes prepared with 5 μg/mL rVN coating solution, and Matrigel-coated dishes after 12 days of induction. Survival rate of HPS0077 hiPSCs is also plotted. *$p < 0.05$.