Electronic Supplementary Information

Vertical nanocolumn-assisted pluripotent stem cell colony formation with minimal cell-penetration

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Materials and Methods

MTS assay

Cell viability was determined using a CellTiter 96 Aqueous One Solution Cell Proliferation assay (MTS assay, Promega), according to the protocols provided by the manufacturer. In brief, various surfaces with cells cultured for 24 h were transferred in 48-well plates containing 20% MTS solution in fresh growth medium and were incubated for 3 h at 37 °C. The change of absorbance at 490 nm was measured using a spectrophotometric plate reader.

3D image visualization

For vSNA labeling experiments, APTMS-treated vSNAs were coated with Alexa Fluor 568 carboxylic acid, succinimidyl ester (Invitrogen), prepared according to the manufacture's recommendation. After 1 h, samples were washed 3 times with distilled, sterile water, and blown dry. iPSCs were stained with Vybrant DiD Cell-Labeling Solution (Molecular Probe) according to the manufacture's recommendation. After 10 min, cells were washed twice with media. The cells were seeded on labeled- vSNAs, and incubated for 30 min. A laser-scanning confocal fluorescence microscope (FV1000, BX61WI, Olympus, Japan) was used to take Z-stacked images of cells on vSNAs. The 3-D images were reconstructed by ImageJ software.

AP activity assay

The AP activity assay was performed according to the manufacturer's instructions. In brief, the cells were washed twice with cold PBS and lysed in Cell Lysis Buffer from a StemTAG AP activity assay kit (Cell Biolabs, USA). After the cells were incubated for 10 min at 4 °C, the lysate was transferred to a fresh tube and centrifuged at 12,000 × *g* for 10 min. Protein concentrations were subsequently determined. Each 50 μL of the cell lysate was transferred

to a 96-well plate in triplicate and mixed with 50 μL of the StemTAG AP activity assay reagent. After incubating for 20 min at 37 °C, the reaction was stopped by adding 50 μL of 1 × stop solution, and the solutions were mixed by placing the plate on an orbital plate shaker for 30 s. The activity was determined by measuring the absorbance at 490 nm and normalizing it to the amount of protein in the reaction.

Table S1. The physical properties of the vSNAs used in this study.

	Diameter (bottom surface, nm)	Density (per 100 μm^2)	Length (μm)
Short	170 ± 4	155 ± 5	1.0 ± 0.010
Medium	160 ± 4	163 ± 24	1.8 ± 0.080
Long	161 ± 7	110 ± 4	4.0 ± 0.36
vSNA1	170 ± 3	29± 2	0.60 ± 0.010
vSNA2	165 ± 4	155 ± 5	1.0 ± 0.010
vSNA3	117 ± 13	396 ± 35	0.70 ± 0.030
vSNA4	136 ± 8	710 ± 2	0.80 ± 0.040

Values are means ± S. D.

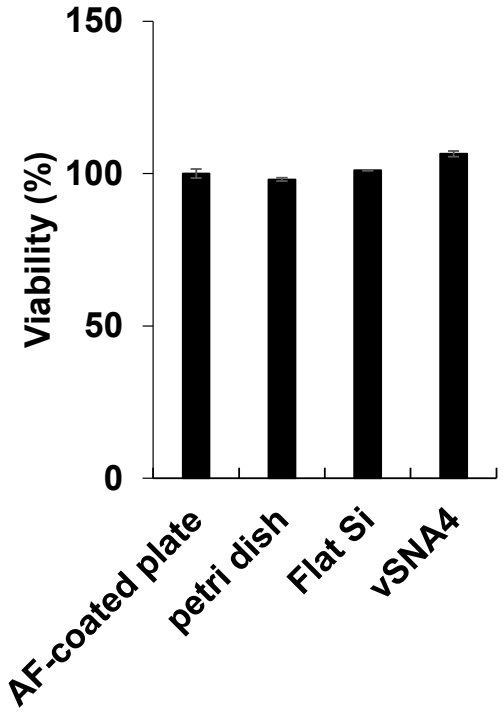

Fig. S1 The effect of vSNAs on cell viability. An MTS assay was performed using iPSCs cultured on various substrates after 24 h of culture.

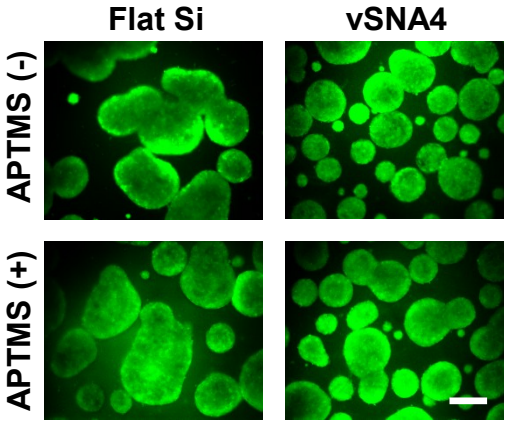

Fig. S2 The effect of APTMS on the formation of spherical colonies. Fluorescence images of spherical iPSC colonies were obtained by CFDA staining. The cells were cultured on flat Si or vSNA4, uncoated or coated with APTMS, for 3 days. Scale bar denotes 200 μm.

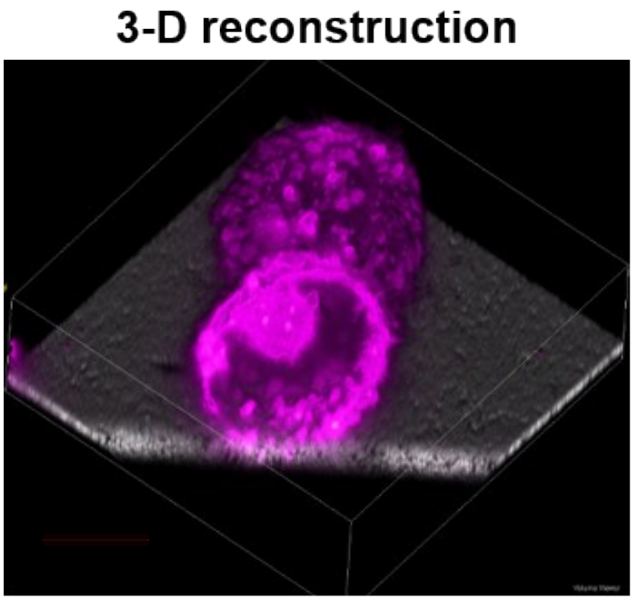

Fig. S3 3-D reconstruction of confocal microscope images. iPSC membrane in magenta; vSNA4 in white. Scale bar denotes 10 μm.

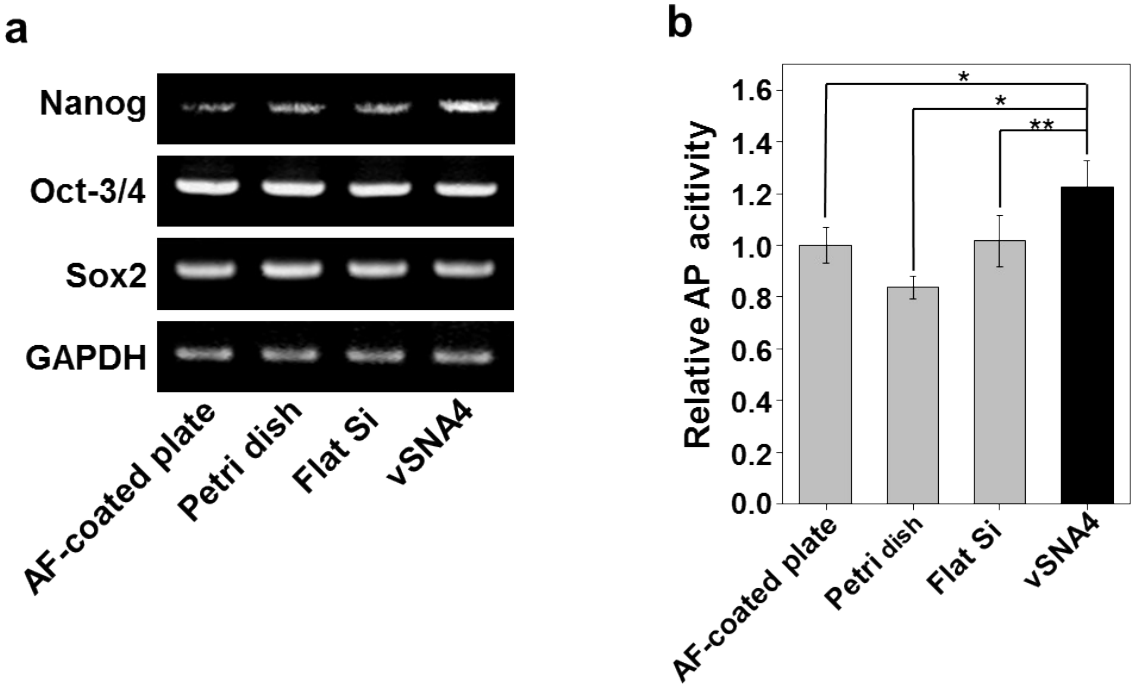

Fig. S4 Expression of pluripotent markers. (a) Semiquantitative RT-PCR analysis of the following pluripotent stem cell markers: Nanog, Oct-3/4, and Sox-2; GAPDH was used as a control. (b) AP activity of iPSCs. $*P < 0.05$; $**P < 0.005$.

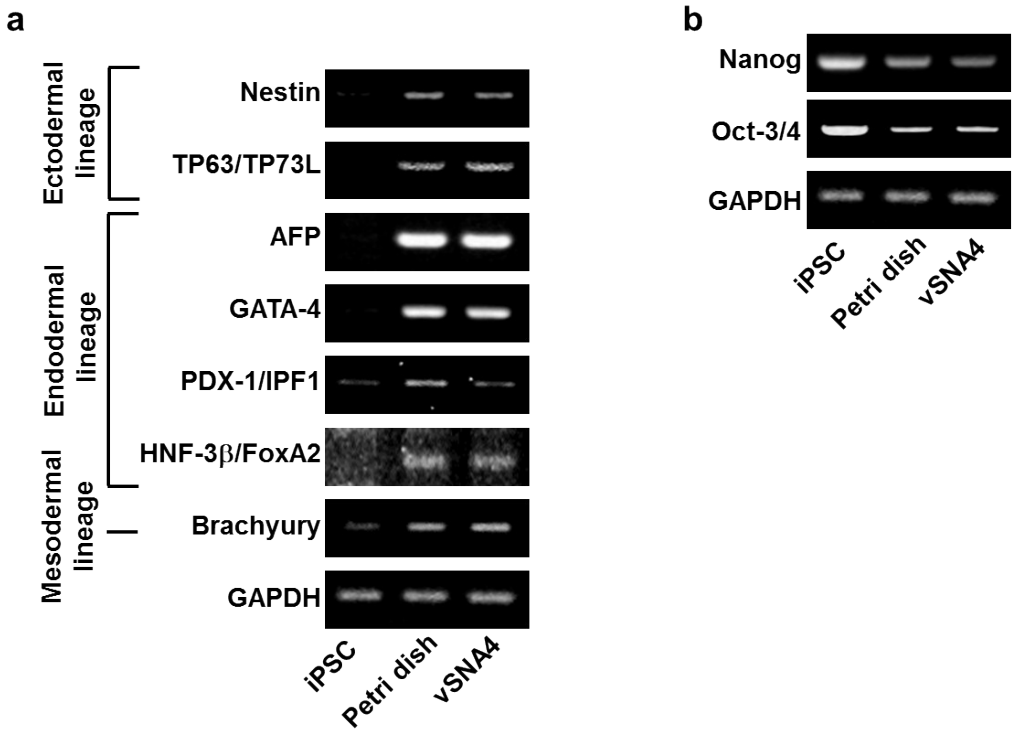

Fig. S5 Differentiation capabilities of iPSCs on vSNA4. (a) Semiquantitative RT-PCR analysis for markers of the three germ layers in iPSCs cultured on petri dishes and vSNA4 in EB medium for 14 days: ectoderm (Nestin and TP63/TP73L), endoderm (AFP, GATA-4, PDX-1/IPF1, and HNF-3β/FoxA2), and mesoderm (Brachyury). (b) Semiquantitative RT-PCR analysis of pluripotent stem cell markers (Nanog and Oct-3/4) in iPSCs grown on various substrates after 14 days of culture.