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Supporting Information

Bioactive calcium silicate extracts regulate the morphology and stemness of human embryonic stem cells at initial stage

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Table S1. Summarized primer sequences of the target genes tested.

Transcript	Gene bank	Primer Sequence	TM (°C)
Oct-4	NM_001285987	F: 5′ GAC AAC AAT GAA AAT CTT CAG GAG A 3′ R: 5′ TTC TGG CGC CGG TTA CAG AAC CA 3′	60
Nanog	XM_011520852	F: 5′ AGC CTC TAC TCT TCC TAC CAC C 3′ R: 5′ TCC AAA GCA GCC TCC AAG TC 3′	60
Bra	XM_011536081	F: 5′ CCA GGT CCC GAA AGA TG 3′ R: 5′ TGC CAA AGT TGC CAA TAC 3′	50
Foxa2	XM_011529231	F: 5′ ACG ACT GTT TCC TGA AGG T 3′ R: 5′ TTG AAG GCG TAG TGG TGT 3′	52
ALP	BC021289	F: 5′ AGC CCT TCA CTG CCA TCC TGT 3′ R: 5′ ATT CTC TCG TTC ACC GCC CAC 3′	65
Runx2	AH005498	F: 5′ TCA CCT TGA CCA TAA CCG TCT 3′ R: 5′ CGG GAC ACC TAC TCT CAT ACT 3′	65
Actin	XM_006715764	F: 5′ TCA CCA CCA CGG CCG AGC G 3′ R: 5′ TCT CCT TCT GCA TCC TGT CG 3′	60

Table S2. Summarized mild apoptosis percentages for hESC H9 cells.[†]

	Apoptosis percentage at 1+3 day	
	Negative	Apoptosis
Con	99.03±0.76%	0.97±1.52%
1/256	99.01±0.84%	0.99±0.84%
1/64	98.31±1.57%	1.69±1.57%

[†]: All colonies were first placed in plain PSCeasy medium for one day, and then cultured in plain, 1/256, or 1/64 CS-supplemented PSCeasy medium for additional three days. TUNEL kit was used to detect, *via* confocal microscopy, the mild apoptosis cells and data were obtained from 15~17 colonies in each cases.

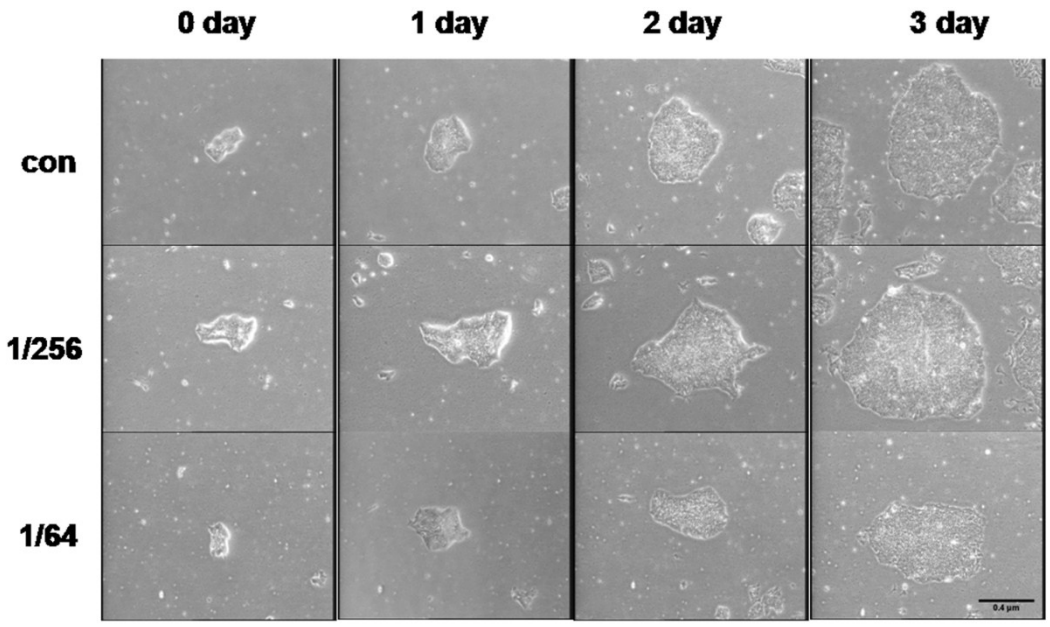

Fig. S1 Typical optical images for hESC H9 colonies on Matrigel. All colonies were first placed in plain PSCeasy medium for one day, and then cultured in plain (*1st row*) 1/256 (*2nd row*), or 1/64 (*3rd row*) CS-supplemented PSCeasy medium for additional three days. Scale bar = 400 μm.

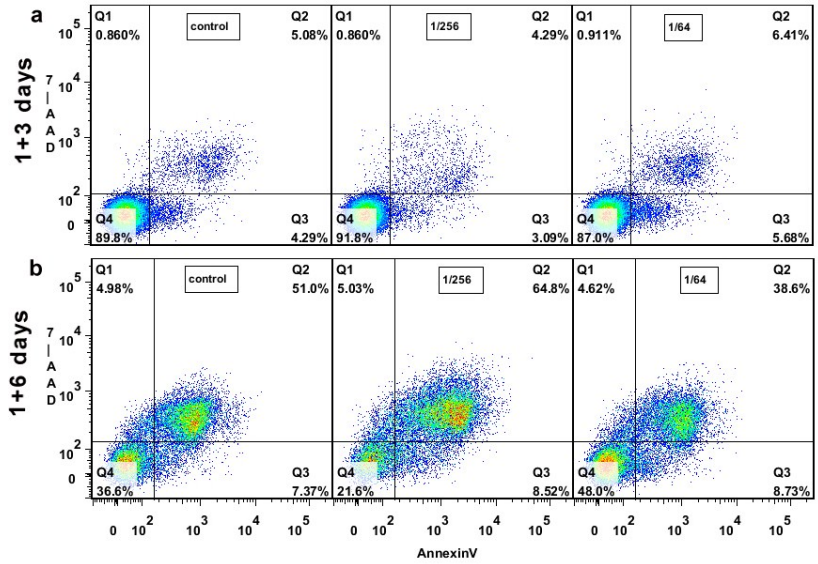

Fig. S2 Apoptosis analysis *via* flow cytometry for hESC H9 colonies on Matrigel. All colonies were first placed in plain PSCeasy medium for one day, and then cultured in plain (*1st column*), 1/256 (*2nd column*), or 1/64 (*3rd column*) CS-supplemented PSCeasy medium for additional three (*1st row*) or six (*2nd row*) days. Each condition has nine replicates.

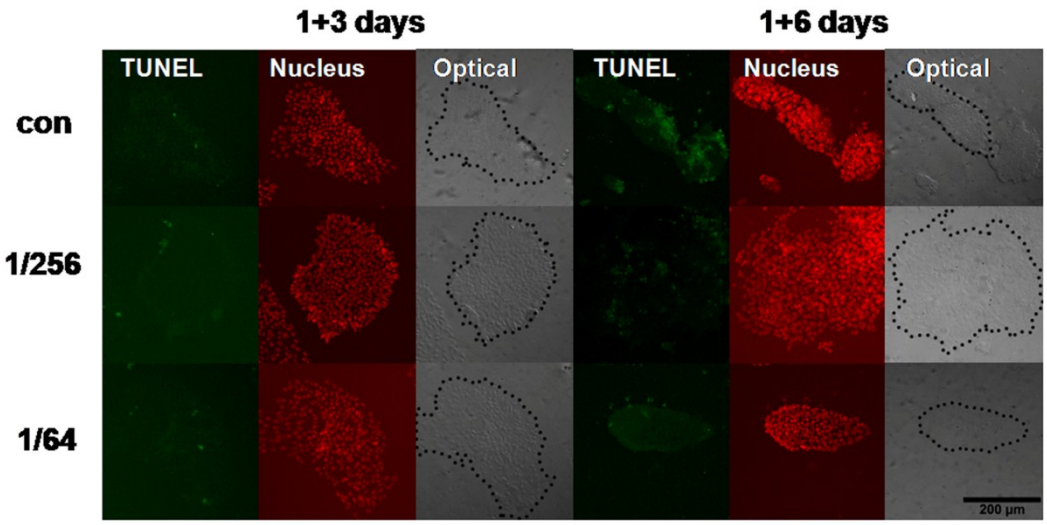

Fig. S3 Typical apoptosis analysis *via* confocal microscopy for hESC H9 colonies on Matrigel. All colonies were first placed in plain PSCeasy medium for one day, and then cultured in plain (*1st row*), 1/256 (*2nd row*), or 1/64 (*3rd row*) CS-supplemented PSCeasy medium for additional three ((1+3) days; *1st-3rd columns*) or six ((1+6) days; *4th-6th columns*) days. Tunnel staining (*1st column*) was visualized under three conditions when nucleus staining (*2nd column*) and optical images (*3rd column*) serve as reference. *Black dotted lines* illustrate the contour of the colonies.

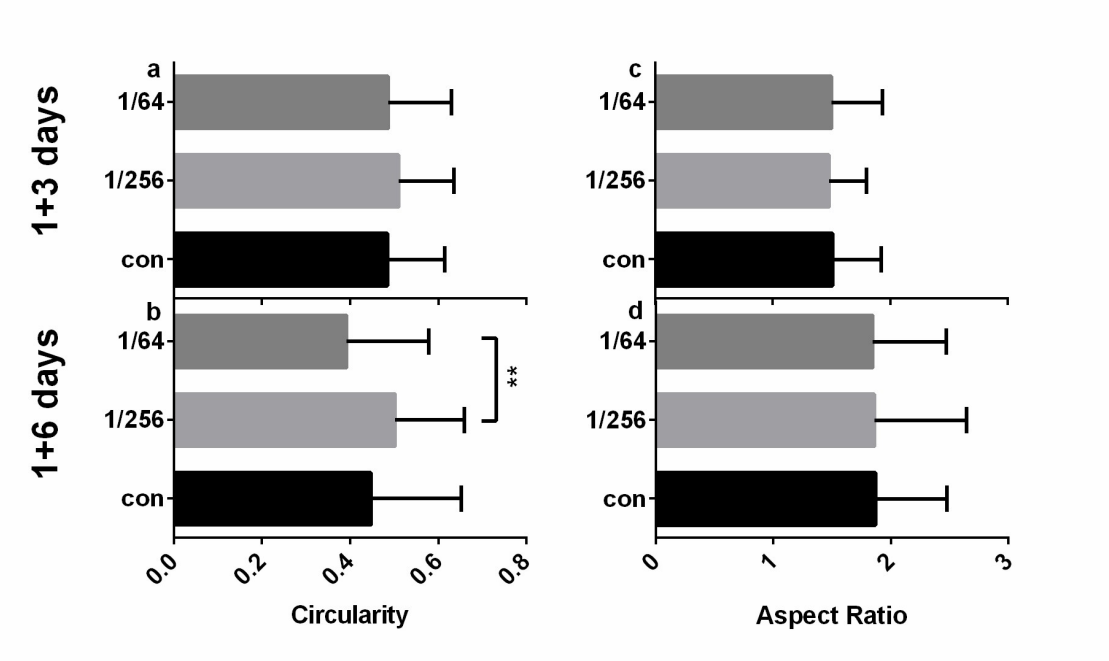

Fig. S4 Morphological analysis of hESC H9 colonies in plain (*black bars*), 1/256 (*light grey bars*), or 1/64 (*dark grey bars*) CS-supplemented PSCeasy medium for short- (*1st row*) or long-period (*2nd row*) culture. Data are obtained from the immunostaining images in three repeated tests and presented as the mean ±SD of colony circularity (*1st column*) and aspect ratio (*2nd column*), respectively, for totally 60 colonies.