SUPPLEMENTARY FIGURES AND TABLES

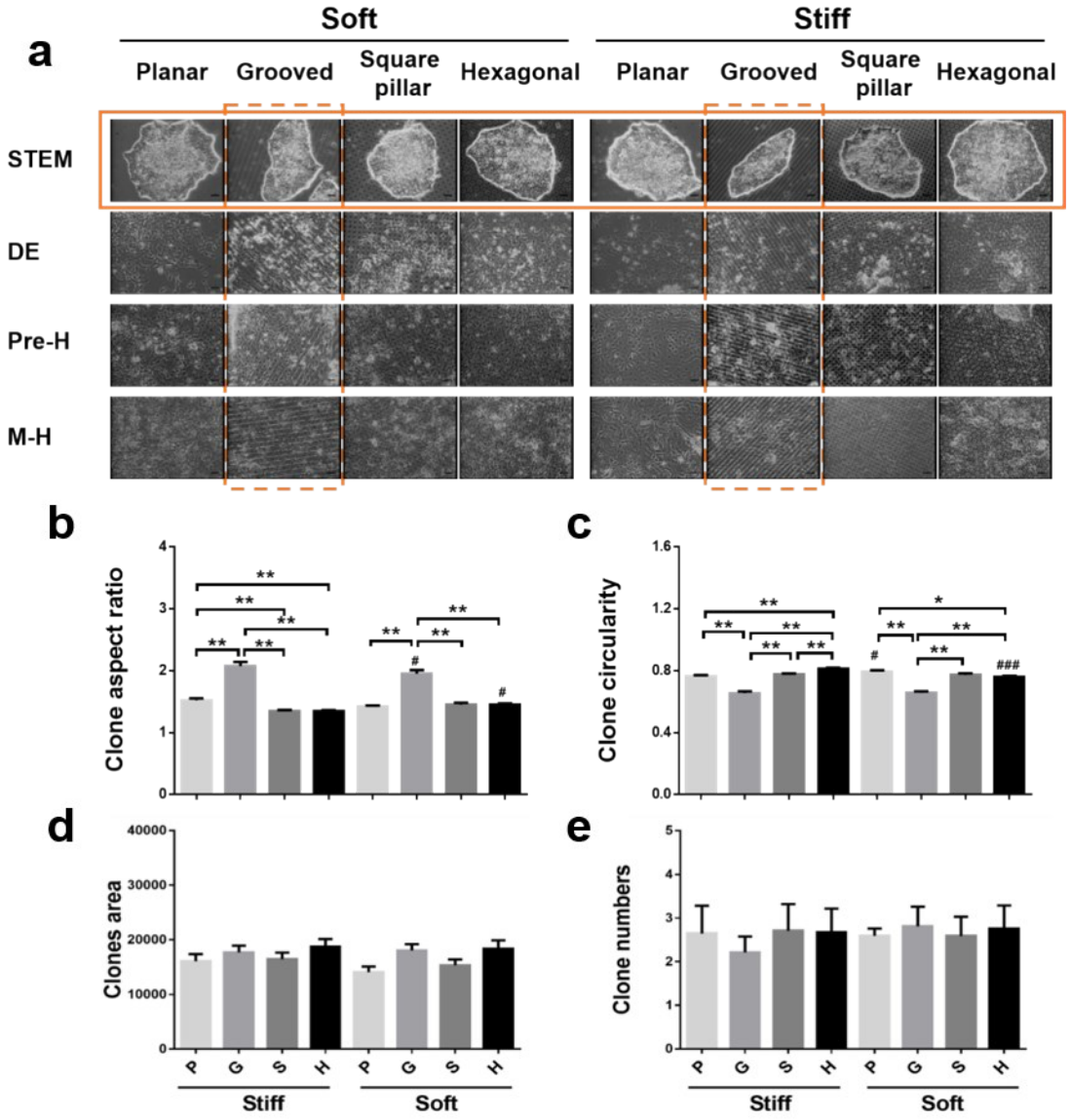

Figure S1. Morphology of H1 cells on PA hydrogel. (a) Optical images of H1 cells on a topographical PA hydrogel pre-coated with 200 μg/ml collagen I in planar (*P*), grooved (*G*), square pillar (*S*), or hexagonal (*H*) configurations, illustrating their morphological changes from stem cells (STEM) through definitive endoderm (DE) and precursor hepatocytes (Pre-H) to hepatocyte-like cells (M-H) on soft (6.1 kPa) or stiff (46.7 kPa) substrates. Bar = 50 μm. *Dotted boxes* indicated the typical morphology on grooved PA gel. (B-E) Morphological analyses of H1 cells on topography- or stiffness-varied substrates. The aspect ratio (b), circularity (c), and projected area (d) of H1

clones were measured (as shown in the *solid box* in panel A) and the average number of clones per unit area was determined in each replicate (E). Data were presented as the mean ± SE from triplicate repeats (totally >132 colonies). * or **, $P < 0.05$ or 0.01 compared with the values between distinct topographies at same stiffness. $^{\#}$ or $^{\#\#\#}$, $P < 0.05$ or 0.001 compared with the values between stiff and soft substrates in same configuration (two-way ANOVA).

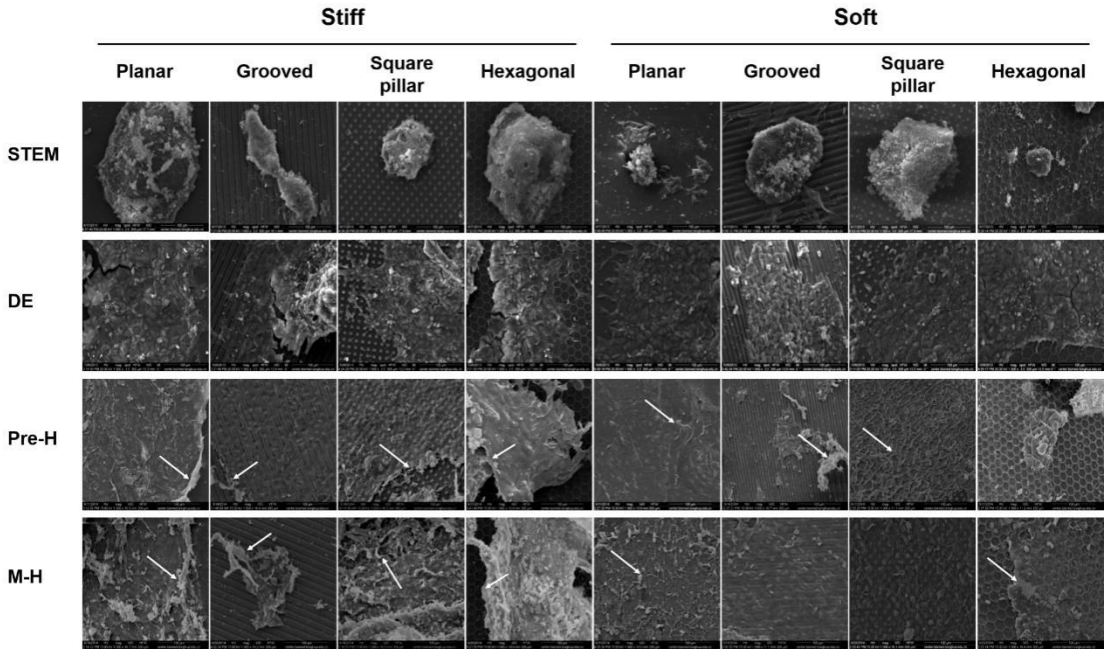

Figure S2. SEM images of H1 cells differentiating into HLCs at the four stages. *Arrows* indicated the sites where ECM tended to be accumulated at the Pre-H or M-H stage.

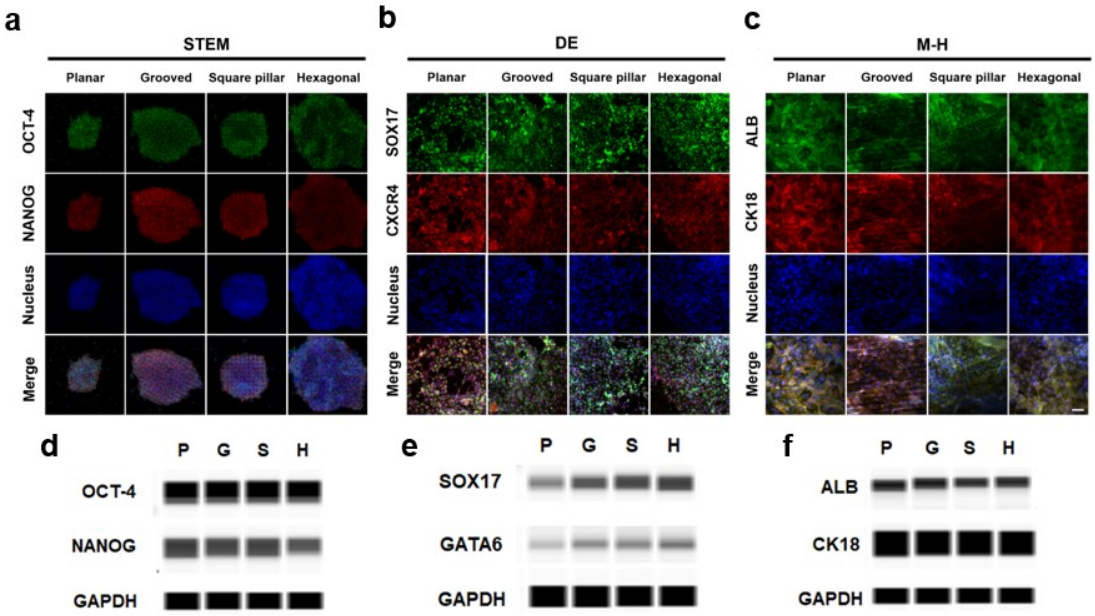

Figure S3. Biomarker expressions of H1 cells at different topographies on *stiff* PA gel. Typical immunostaining (*1st row*) or immunoblotting (*2nd row*) images of OCT-4 and NANOG (a, d), SOX17 and CXCR4 (or GATA6) (b, e), or ALB and CK18 (c, f) were illustrated at the STEM, DE or M-H stage. Bar = 50 μm.

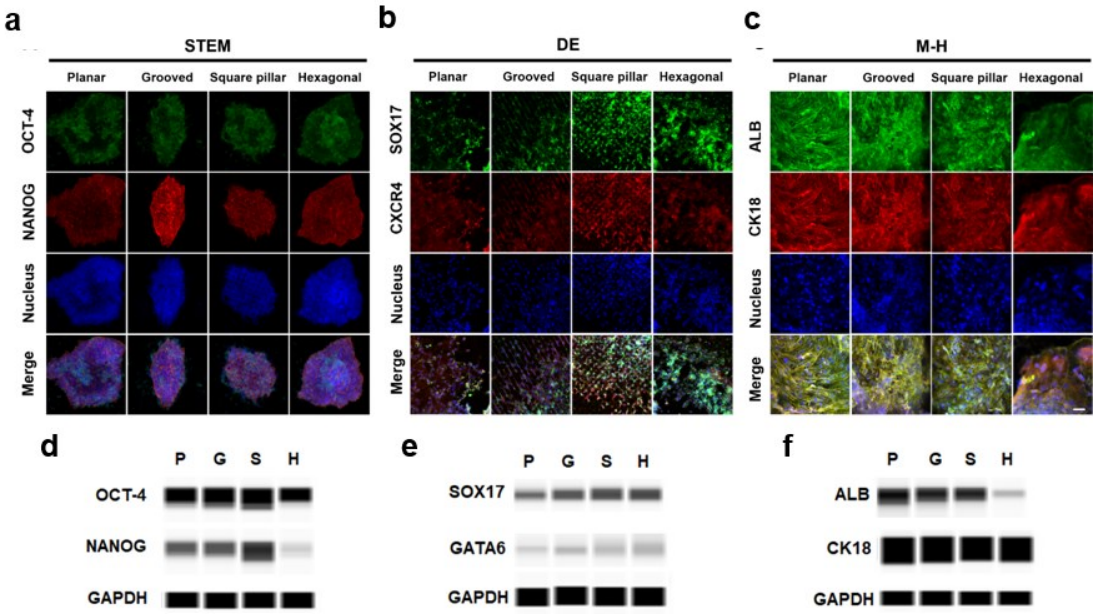

Figure S4. Biomarker expressions of H1 cells at different topographies on *soft* PA gel. Typical immunostaining (*1st row*) or immunoblotting (*2nd row*) images of OCT-4 and NANOG (a, d), SOX17 and CXCR4 (or GATA6) (b, e), or ALB and CK18 (c, f) were illustrated at the STEM, DE or M-H stage. Bar = 50 μm.

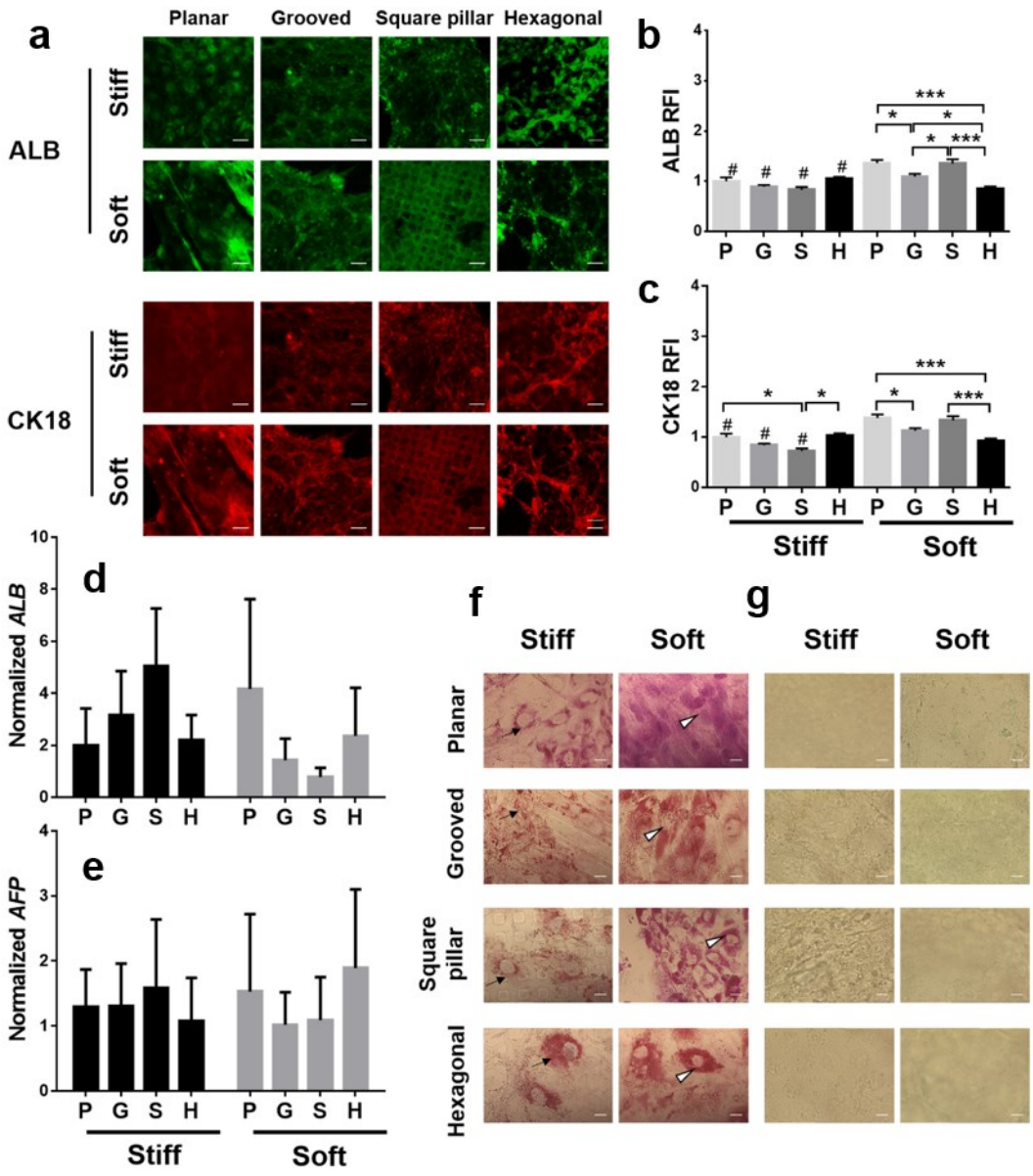

Figure S5. Functional tests of the differentiated H1 cells at the Pre-H stage. (a-e) Typical immunostaining images (a) and the related quantifications of ALB (b) and CK18 (c) biomarkers, as well as *ALB* (d) and *AFP* (e) gene expressions were presented. (f-g) Also plotted were the histological and histochemical staining of glycogen synthesis (f) and ICG engulfment (g). *Arrows* or *arrowheads* indicated the differences on a stiff or soft substrate, respectively. * or ***, $P < 0.05$ or 0.001 compared with the values between distinct topographies at same stiffness. $^{\#}$, $P < 0.05$ compared with the values between stiff and soft substrates in same configuration (two-way ANOVA).

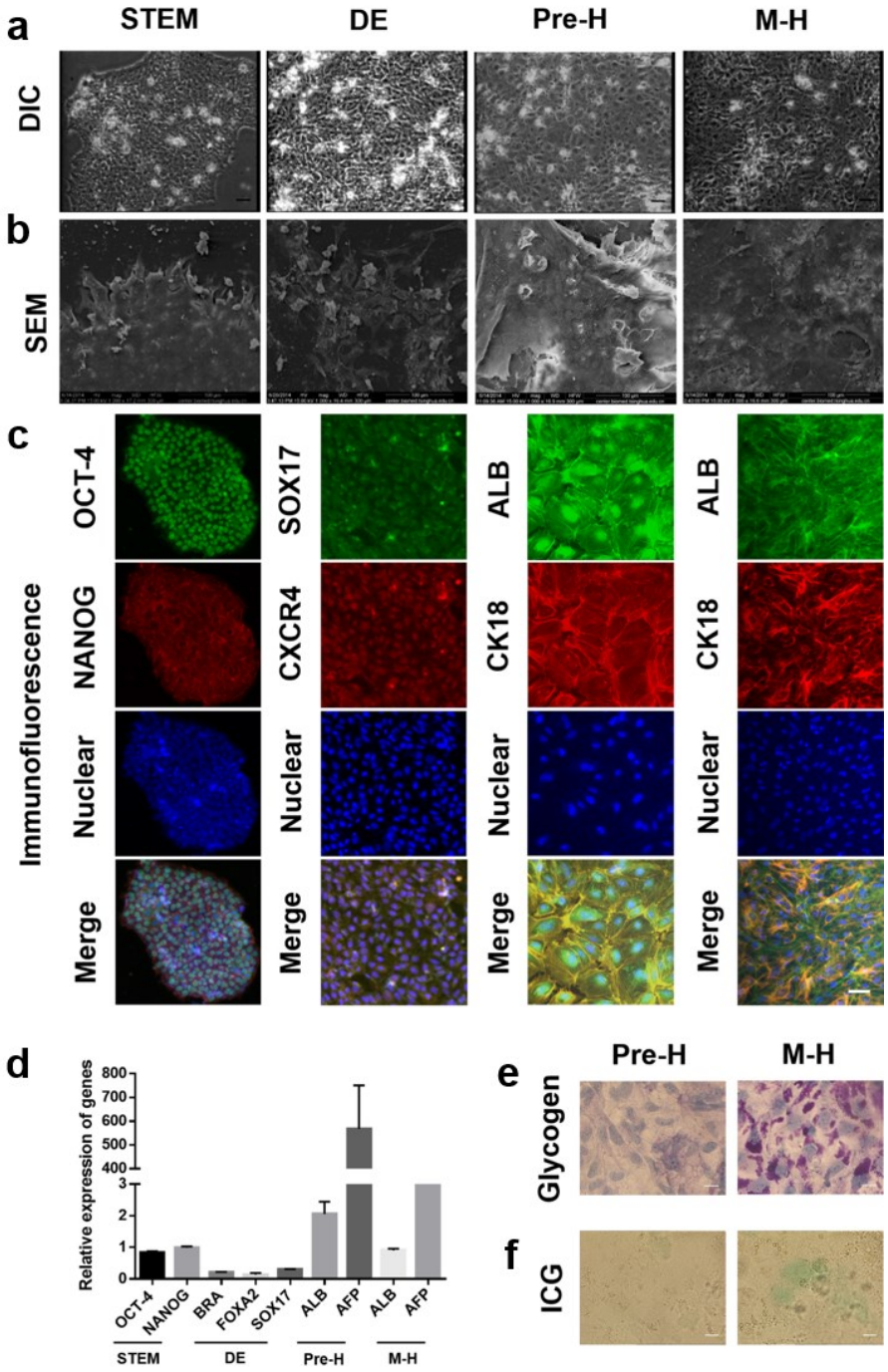

Figure S6. Summaries of growing H1 cells on matrigel-coated petri dish. (a-b) Typical optical (a) and SEM (b) images of H1 cells at the four stages. (c) Typical biomarker immunostaining of OCT-4 and NANOG at the STEM, SOX17 and CXCR4 at the DE, and ALB and CK18 at the Pre-H or M-H stage. Bar = 50 μm. (d) Related gene expressions of *OCT-4* and *NANOG* at the STEM, *BRA*, *FOXA2* and *SOX17* at the DE, and *ALB* and *AFP* at the Pre-H or M-H stage. (e-f) Histological and histochemical staining of glycogen synthesis (E) and ICG engulfment (F). Bar = 50 μm.

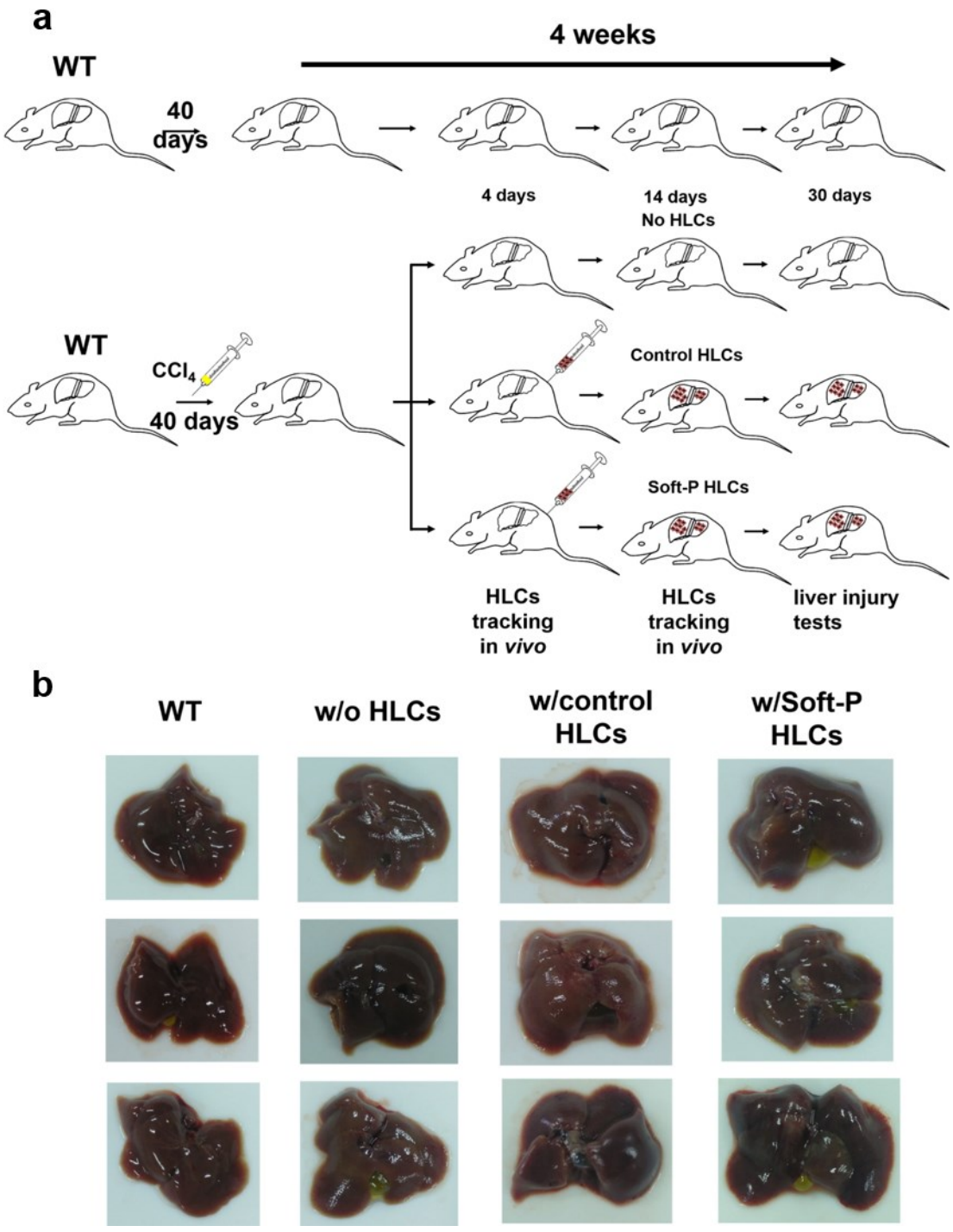

Figure S7. Experimental procedures and optical liver images in mice. (a) Schematic of preparing the mice with CCl_4-induced liver injury and HLCs transplantation. (b) Optical liver images of WT mice, CCl_4-treated mice, and CCl_4-treated mice with transplanted control or Soft-P HLCs.

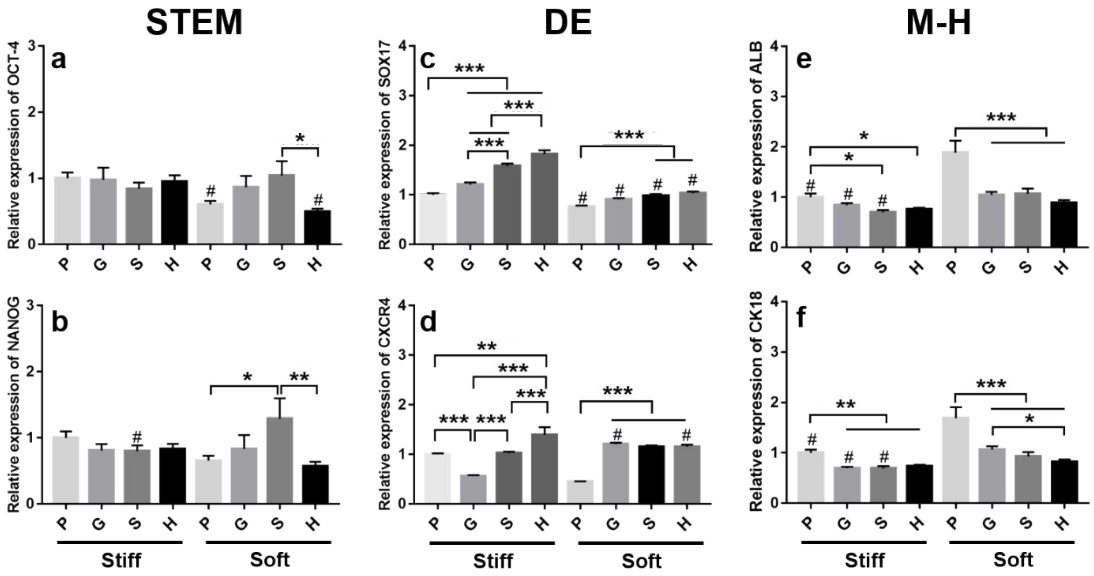

Figure S8. Distinct impacts of substrate topography and stiffness on pluripotency maintenance of hESCs at the Stem stage (a, b), differentiation stage-specific marker expression at the DE (c, d) and M-H stage (e, f). Relative fluorescent intensity (RFI) of two stemness biomarkers of OCT-4 (a) or NANOG (b) was quantified and presented as the mean ± SE for > 64 clones. The RFI values of SOX17 (c) and CXCR4 (d) were quantified for > 30 fields of view (FOVs) in triplicate. Data are presented as the mean ± SE of > 200 individual cells. The RFI values of ALB (e) and CK18 (f) were presented as the mean ± SE for > 21 FOVs in triplicate. *, ** or ***, $P < 0.05$, 0.01 or 0.001 compared with the values between distinct topographies at same stiffness; $^{\#}$: $P < 0.05$ compared with the values between stiff and soft substrates in the respective configuration (two-way ANOVA).

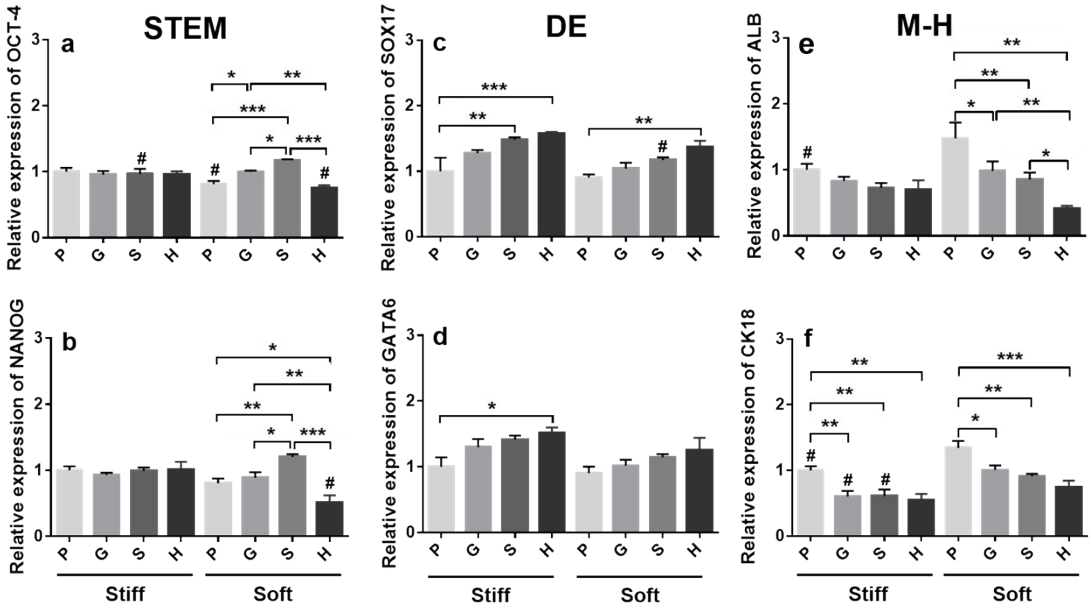

Figure S9. Distinct impacts of substrate topography and stiffness on pluripotency maintenance of hESCs at the Stem stage (a, b), differentiation stage-specific marker expression at the DE (c, d) and M-H stage (e, f) by Immunoblotting. Two stemness biomarkers of OCT-4 (a) or NANOG (b) was quantified and presented as the mean ± SE from five repeats. The values of SOX17 (c) and CXCR4 (d) were quantified from five repeats. Data are presented as the mean ± SE. The values of ALB (e) and CK18 (f) were presented as the mean ± SE for from ten repeats. *, ** or ***, $P < 0.05$, 0.01 or 0.001 compared with the values between distinct topographies at same stiffness; $^{\#}$: $P < 0.05$ compared with the values between stiff and soft substrates in the respective configuration (two-way ANOVA).

Table S1. All the primer sequences and annealing temperatures for all the genes tested.

Name	Primer sequence
OCT-4	5’-GACAACAATGAAAATCTTCAGGAGA-3’
	5’-TTCTGGCGCCGGTTACAGAACCA-3’
NANOG	5’-AGCCTCTACTCTTCCTACCACC-3’
	5’-TCCAAAGCAGCCTCCAAGTC-3’
SOX17	TTCGTGTGCAAGCCTGAGAT
	TAATATACCGCGGAGCTGGC
CXCR4	AGTGATAAACACGAGGATGGCAAG
	TGTATATCTCCTCCCCCAAGCG
BRA	5’-CCAGGTCCCGAAAGATG-3’
	5’-TGCCAAAGTTGCCAATAC-3’
FOXA2	5’-ACGACTGTTTCCTGAAGGT-3’
	5’-TTGAAGGCGTAGTGGTGT-3’
GATA6	5’-CCATGACTCCAACTTCCACC-3’
	5’-ACGGAGGACGTGACTTCGGC-3’
ALB	AGCCTTGGTGTTGATTGCCT
	CTCTGGTCTCACCAATCGGG
CK18	AAATCCGGGAGCACTTGGAG
	CAATCTGCAGAACGATGCGG
AFP	5’-TTACACAAAGAAAGCCCC-3’
	5’-TCCGATAATAATGTCAGCC-3’
GAPDH	5’-GGTGAAGGTCGGAGTCAACGGA-3’
	5’-GAGGGATCTCGCTCCTGGAAGA-3’
ACTIN	5’-TCACCACCACGGCCGAGCG-3’
	5’-TCTCCTTCTGCATCCTGTCG-3’

Table S2. Two Way ANOVA statistical tests for coupling impacts of substrate stiffness and topography[†].

Source of Variation	STEM									DE						M-H		
	OCT-4			NANOG			SOX17			GATA			CXCR4			ALB		
	WES	IF	qPCR	WES	IF	qPCR	WES	IF	qPCR	WES	IF	qPCR	WES	IF	qPCR	WES	IF	qPCR
Stiffness	0.307	0.045	0.020	0.021	0.825	<0.001	0.003	<0.001	0.092	0.008	0.926	0.780	0.193	<0.001	<0.001	<0.001	<0.001	0.045
Topography	<0.001	0.306	0.308	0.002	0.138	0.660	<0.001	<0.001	0.187	0.004	<0.001	0.659	<0.001	<0.001	<0.001	<0.001	<0.001	0.067
Stiffness ⊕ Topography	<0.001	0.059	0.122	<0.001	0.023	0.637	0.712	<0.001	0.836	0.827	<0.001	0.523	0.031	<0.001	<0.001	0.649	<0.001	0.943

[†]: Additional statistical data for any paired cases were not shown for clarity. For details, refer to Figs. 5 and 6 with a distinct normalization of the experimental data.

□: *P* values estimated from Two Way ANOVA analysis.