The Suppression of Medium Acidosis Improves the Maintenance and Differentiation of Human Pluripotent Stem Cells at High Density in Defined Cell Culture Medium

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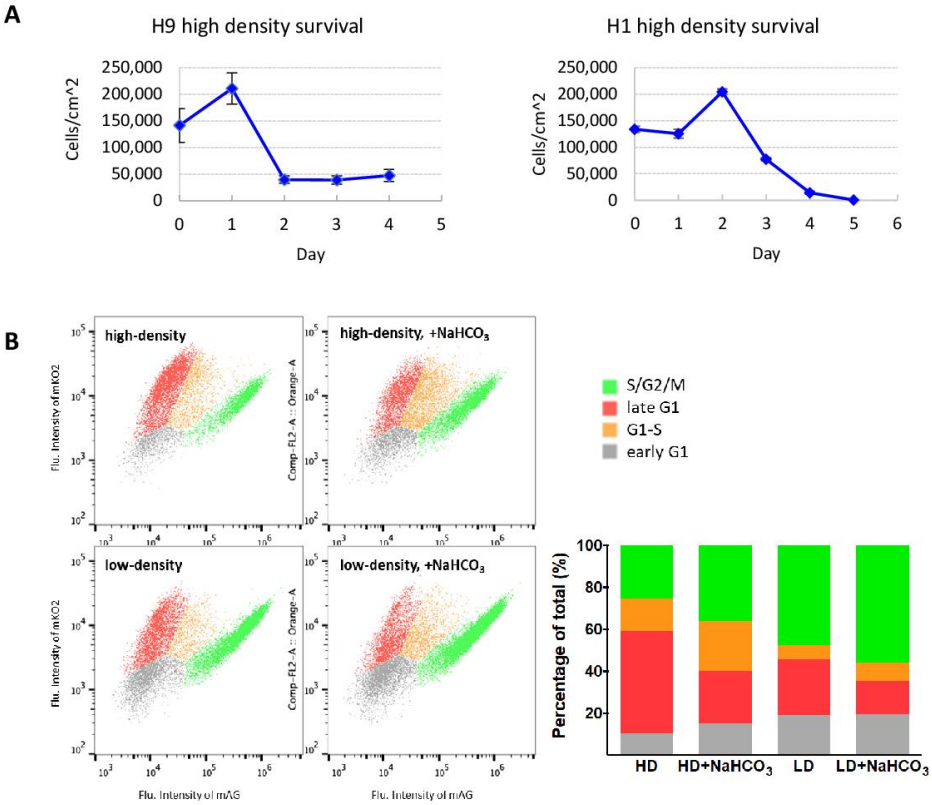
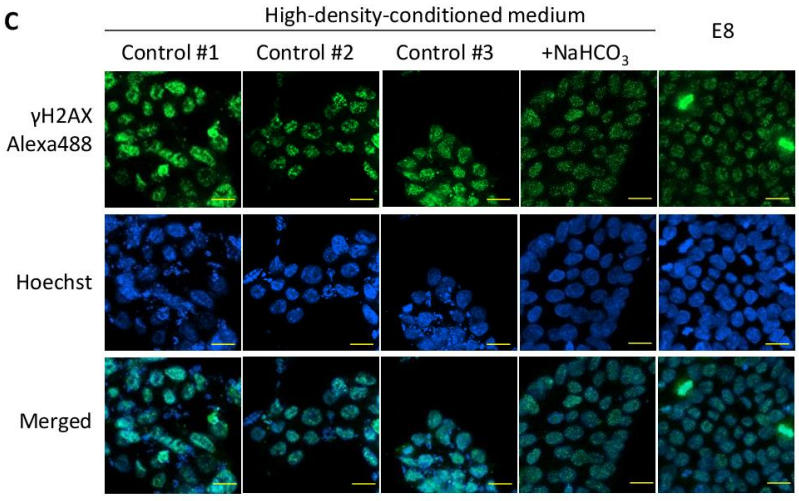
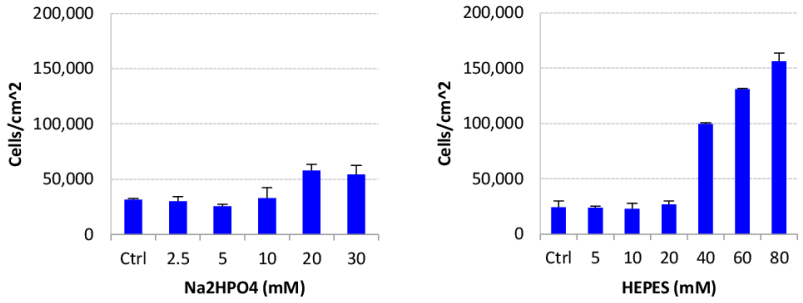
Supplementary Figure 1.





Supplementary Figure 1.

D



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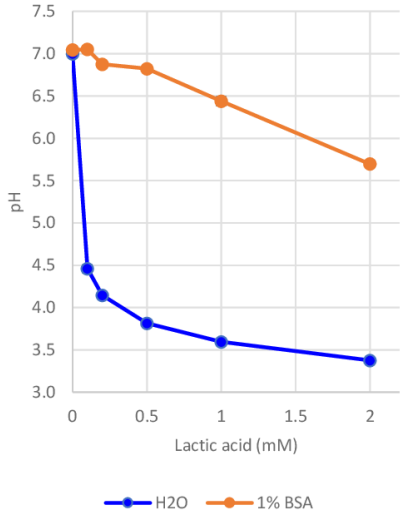

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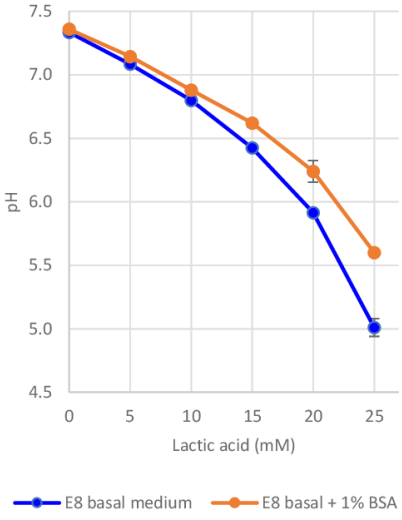
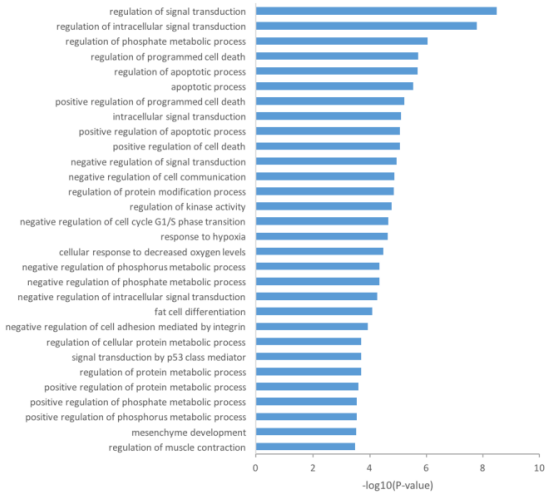


Supplementary Figure 1. ESC cell death at high density due to medium acidosis, and the rescuing effect of pH modulation

(A) Growth curves of H9 and H1 cells plated at high density (n=3). Medium was changed daily. (B) Cell cycle analysis of H1 using FUCCI reporter system to confirm results of Edu-PI study. (C) Anti-phosphorylated γH2A.X immunostaining of H9 cells treated in high-density-conditioned media for 6hrs with or without $NaHCO_3$, compared with control cells maintained in E8. Scale bar, 20 μm. (D) 24-hr cell count of H9 high-density cells cultured with or without Na_2HPO4 or HEPES supplementation (n=3). (E) Pluripotency markers of H1 cells passaged with or without 20mM $NaHCO_3$ treatment for 6 passages, analyzed by real time PCR. (F) pH changes with increasing concentrations of lactic acid added to H_2O versus 1% BSA. (G) pH changes with increasing concentrations of lactic acid added to E8 basal medium with or without 1% BSA.

Supplementary Figure 2.

A



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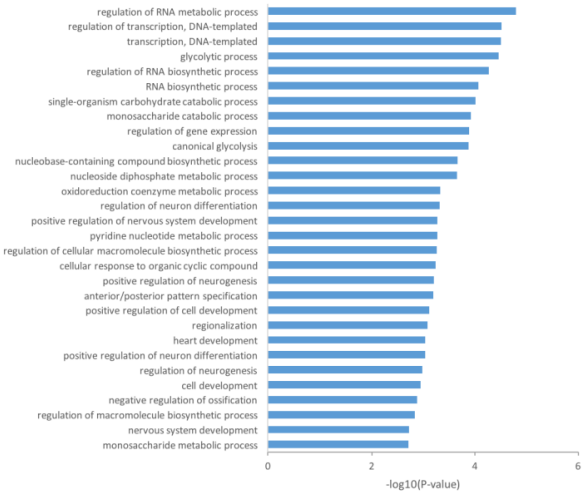
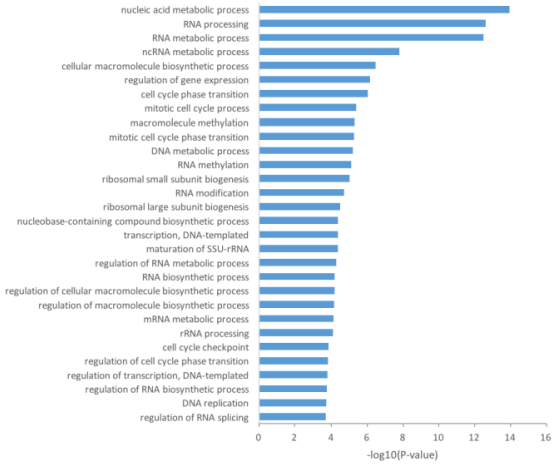
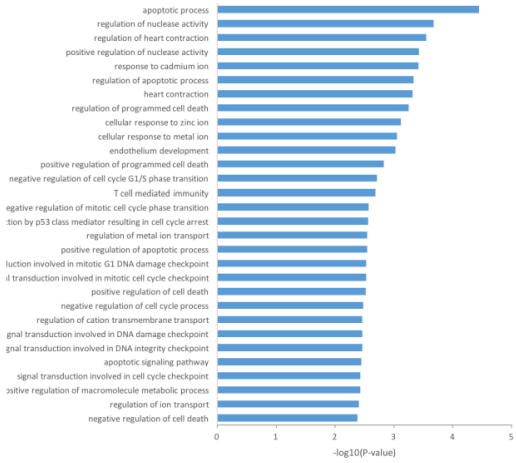


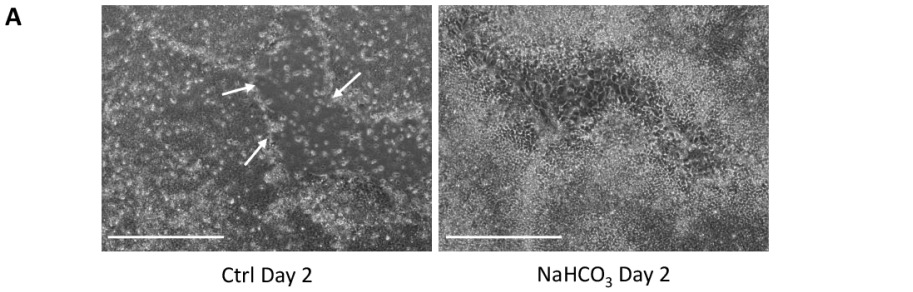




Supplementary Figure 2. Gene expression changes in response to culture density and medium pH

(A) Gene ontology (GO) biological process analysis of the genes upregulated (top) and downregulated (bottom) in high-density compared to low-density culture. (B) GO biological process analysis of the gene upregulated (top) and downregulated (bottom) in $NaHCO_3$-treated compared to untreated high-density culture.

Supplementary Figure 3.



Supplementary Figure 3. Applications of pH modulation in hESC culture maintenance and cardiac differentiation

(A) Representative images of control and $NaHCO_3$-treated H9 cells on day 2 of differentiation. Arrows point to the edge of colonies in control cells, which is not seen in $NaHCO_3$-treated cells due to epithelial–mesenchymal transition. Scale bar, 400 μm. (B) Real-time PCR analysis of early mesoderm markers MIXL1 and T on day 2 and day 3 of H9 differentiation. Expression levels are normalized to that of control cells on day 2. Data are representative of > 3 independent experiments. (C-E) Real-time PCR analysis of cardiac progenitor markers NKX2.5 and TNNT2 on day 10 of H9 (panel C), H1 (panel D), and iPSC line ND1 (panel E) differentiation. Expression levels are normalized to that of control cells. Data are representative of > 3 independent experiments.

Supplemental Table 1. Media component and pH analysis.

	pH	Glucose (g/L)	Glutamine (mmol/L)	Lactate (mmol/L)
Fresh E8 media	7.23 ± 0.04	2.90 ± 0.07	2.05 ± 0.22	0
Media from low-density culture	7.22 ± 0.04	2.69 ± 0.14	1.87 ± 0.26	4.77 ± 1.68
Media from high-density culture	6.40 ± 0.08	1.64 ± 0.09	0.89 ± 0.08	15.19 ± 1.23

Supplemental Table 2. Primers used in real-time PCR. Related to Experimental Procedures.

Gene	Forward primer	Reverse primer
GAPDH	GTGGACCTGACCTGCCGTCT	GGAGGAGTGGGTGTCGCTGT
T	TGTCCCAGGTGGCTTACAGATGAA	GGTGTGCCAAAGTTGCCAATACAC
MIXL1	AGCTGCTGGAGCTCGTCTTC	TGGAAGGATTTCCCACTCTG
NKX2.5	CAAGTGTGCGTCTGCCTTT	CAGCTCTTTCTTTTCGGCTCTA
TNNT2	TTCACCAAAGATCTGCTCCTCGCT	TTATTACTGGTGTGGAGTGGGTGTGG