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Supplementary Materials for

Control of matrix stiffness promotes endodermal lineage specification by regulating SMAD2/3 via lncRNA LINC00458

Yu-Fan Chen, Yi-Shuan J. Li, Chih-Hung Chou, Men Yee Chiew, Hsien-Da Huang, Jennifer Hui-Chun Ho*, Shu Chien*, Oscar K. Lee*

*Corresponding author. Email: jenniferho@ym.edu.tw (J.H.-C.H.); shuchien@eng.ucsd.edu (S.C.); oscarlee9203@gmail.com (O.K.L.)

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The PDF file includes:

Fig. S1. Pluripotency markers are down-regulated in soft substrate.
Fig. S2. GO terms highlighting the characteristics of down-regulated genes in the soft substrates compared to that with hard substrates.
Fig. S3. Combination of soft substrate and chemical factors enhance the urea production of hPSC-derived hepatocytes.
Fig. S4. Most of the differentially regulated lncRNAs show a stiffness-associated expression signature in hPSCs.
Fig. S5. Knockdown of lncRNA *LINC00458* does not affect pluripotency genes.
Fig. S6. The lncRNA *LINC00458* does not act in a cis-regulatory manner.

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/6/eaay0264/DC1)

Movie S1 (.mov format). Morphology of hPSCs upon soft substrate.
Movie S2 (.mov format). Morphology of hPSCs upon hard substrate.
Movie S3 (.mov format). Morphology of hPSCs upon TCPS.

Supplementary Figure 1

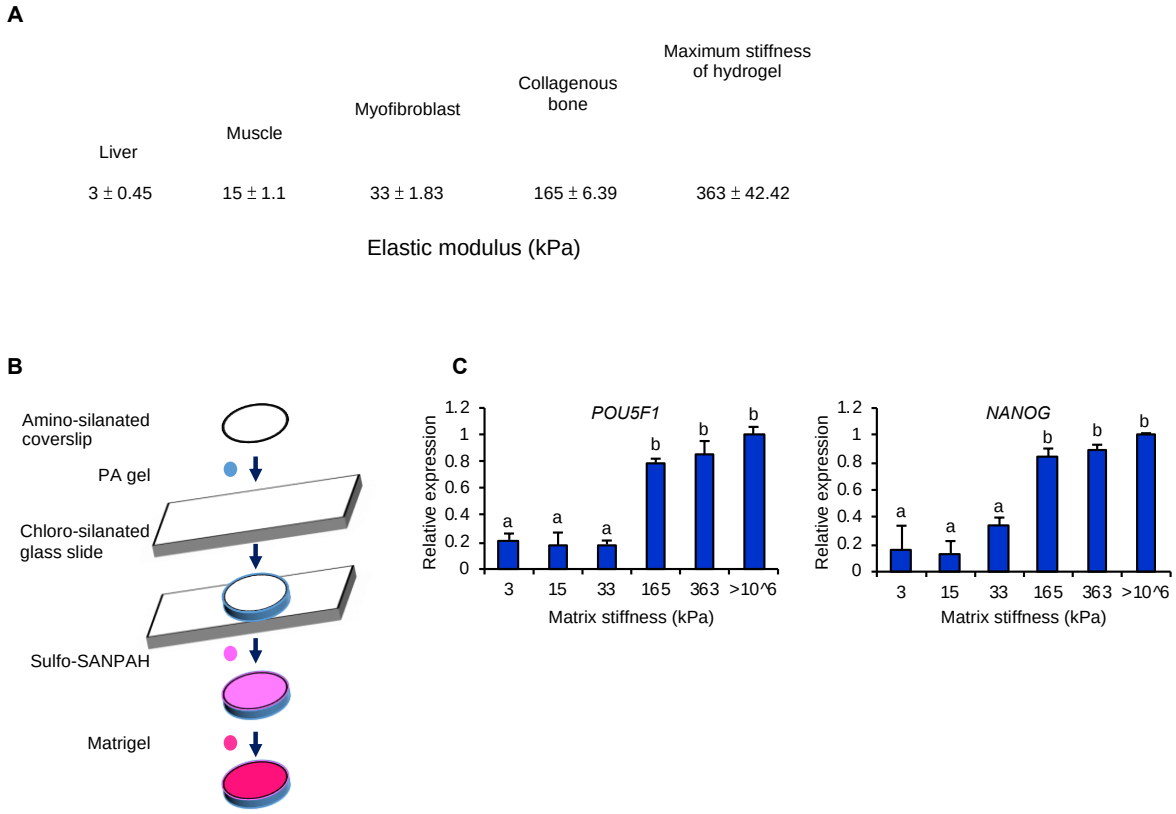

Fig. S1. Pluripotency markers are down-regulated in soft substrate. The elasticity of various human tissues was compared to the defined substrate stiffnesses of polyacrylamide (PA) gels. (B) A schematic diagram of the setup for PA gels. (C) mRNA expression levels of *POU5F1* and *NANOG* were downregulated on soft substrates (3 kPa) compared to those on hard substrates (165 kPa) and tissue culture polystyrene surfaces (TCPS, > 10^6 kPa). The results are expressed as mean ± SD of triplicates. One-way ANOVA (n=3 independent experiments). Different letters indicate significant differences, and the same letters indicate no significant difference.

Supplementary Figure 2

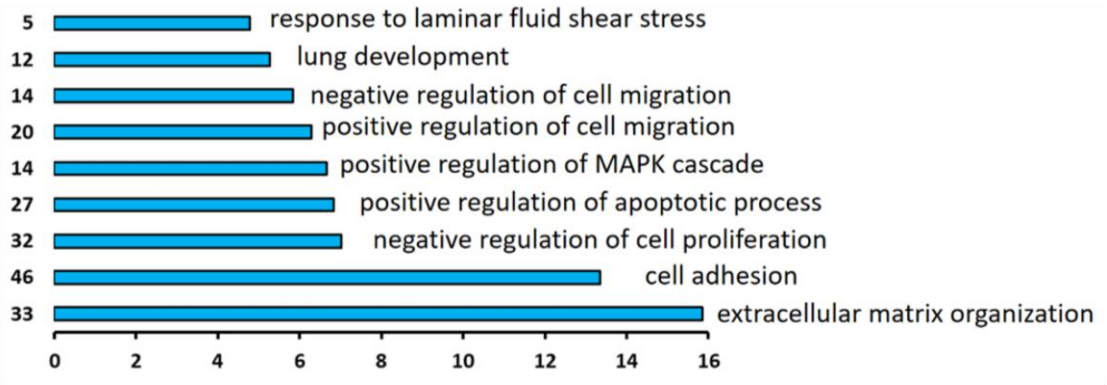

Fig. S2. GO terms highlighting the characteristics of down-regulated genes in the soft substrates compared to that with hard substrates.

Supplementary Figure 3

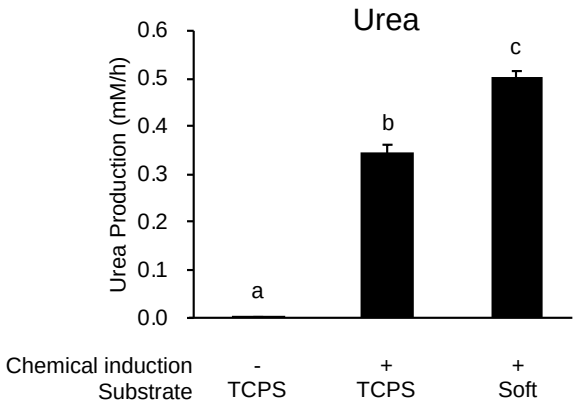

Fig. S3. Combination of soft substrate and chemical factors enhance the urea production of hPSC-derived hepatocytes. Urea production analysis of hPSCs with hepatic differentiation induction for 12 days cultured on tissue culture polystyrene (TCPS, > 10^6 kPa) or soft substrates (3 kPa) supported that soft substrates facilitated hepatocyte differentiation and maturation than TCPS. The results are presented as mean ± SD of triplicates. One-way ANOVA (n=3 independent experiments). Different letters indicate statistically significant differences between groups.

Supplementary Figure 4

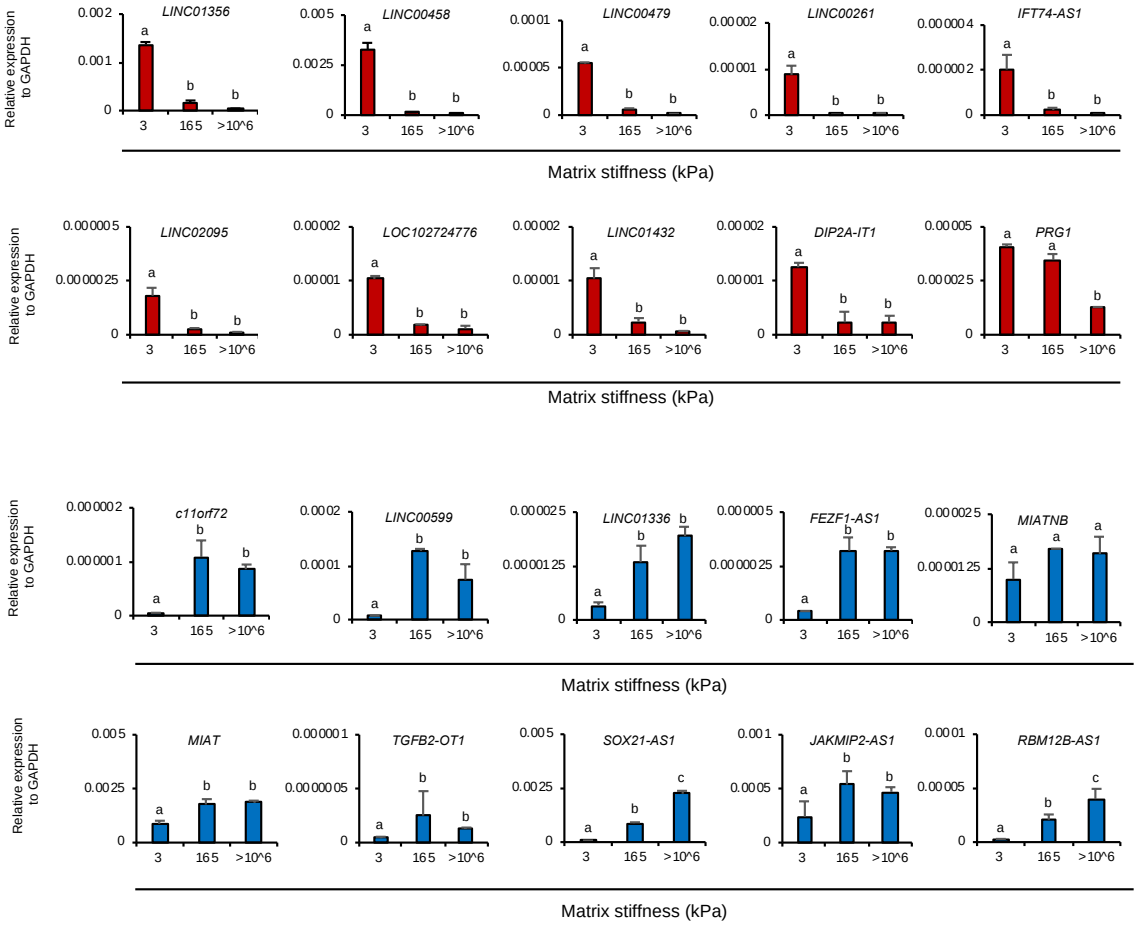

Fig. S4. Most of the differentially regulated lncRNAs show a stiffness-associated expression signature in hPSCs. Top 20 differentially regulated lncRNAs, including (A) 10 upregulated and (B) 10 downregulated, as detected by quantitative RT-PCR with various substrate stiffnesses. The results are expressed as mean ± SD of triplicates. One-way ANOVA (n=3 independent experiments). Different letters indicate significant differences, and the same letters indicate no significant difference.

Supplementary Figure 5

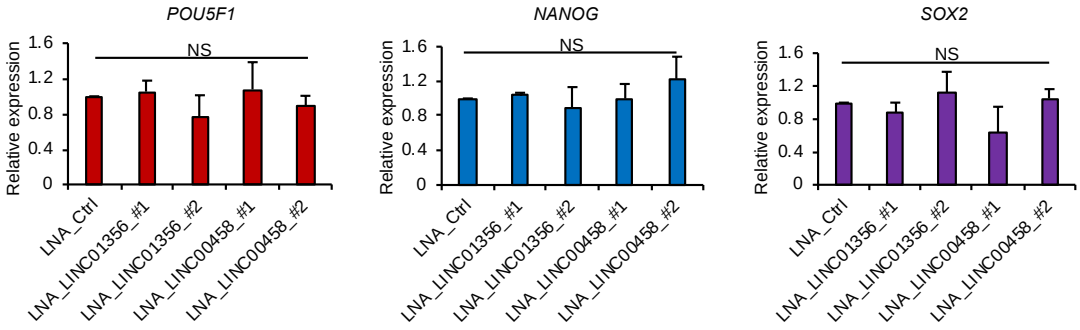

Fig. S5. Knockdown of lncRNA *LINC00458* does not affect pluripotency genes. Human pluripotent stem cells (hPSCs) were cultured on TCPS with LNA GampeRs targeting *LINC00458*, and the pluripotency markers, *POU5F1*, *NANOG*, and *SOX2*, were analyzed by quantitative RT-PCR. NS, Not significant.

Supplementary Figure 6

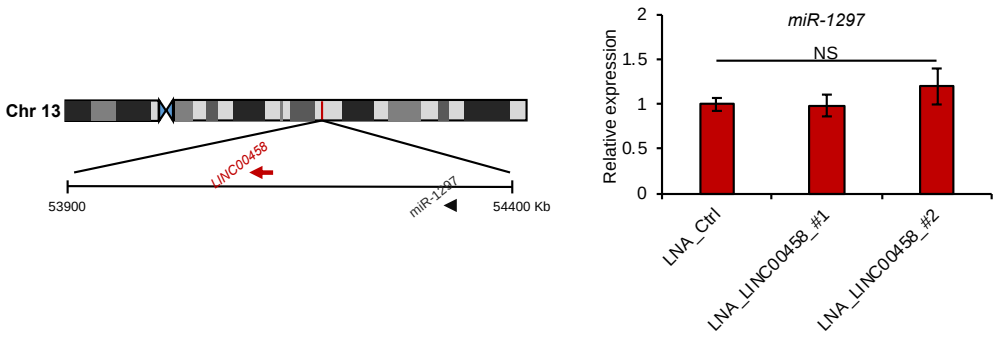

Fig. S6. The lncRNA *LINC00458* does not act in a cis-regulatory manner. Human pluripotent stem cells (hPSCs) were cultured on TCPS with LNA GampeRs targeting *LINC00458*, and *miR-1297* expression was analyzed by quantitative RT-PCR. NS, Not significant.