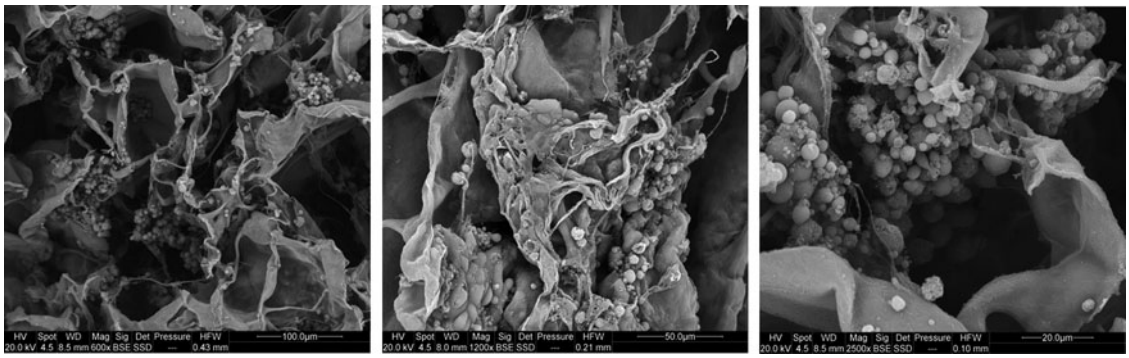
Supplementary Data

SEM Images of Cell-Embedded Scaffold

We captured scanning electron microscope (SEM) images of the mouse embryonic stem cell (mESC)–embedded collagen scaffold 4 days postseeding. In addition to Figure 2 (in the article), higher-magnification images showing the embryoid body (EB) structures within the scaffold pores are presented here.



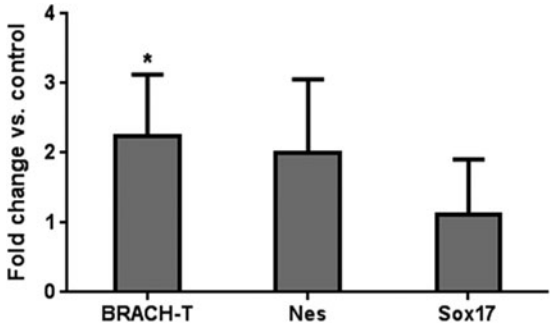
SUPPLEMENTARY FIG. S1. Collagen scaffold scanning electron microscopic (SEM) images.

Examined Genes Representative of the Three Germ Layers

Supplementary Table S1. Genes Analyzed for Each Germ Layer

Germ layer	*Gene*	*Applied Biosystems assay*	*Amplicon length*
Primitive streak	Bry (T, brachyury homolog)	Mm00436877_m1	86
Ectoderm	*Pax6* (paired box 6)	Mm00443072_m1	56
	Nestin	Mm00450205_m1	72
Mesoderm	*Flk1* (*fetal liver kinase 1*)	Mm01222421_m1	64
Endoderm	*Sox17* (sex determining region Y-box 17)	Mm00488363_m1	86
Control	*GAPDH* (glyceraldehyde 3-phosphate dehydrogenase)	Mm99999915_g1	107

Real-Time Polymerase Chain Reaction Analysis on Day 2



SUPPLEMENTARY FIG. S2. Gene expression of mouse embryonic stem cells (mESCs) 2 days postseeding under cyclic stretching.

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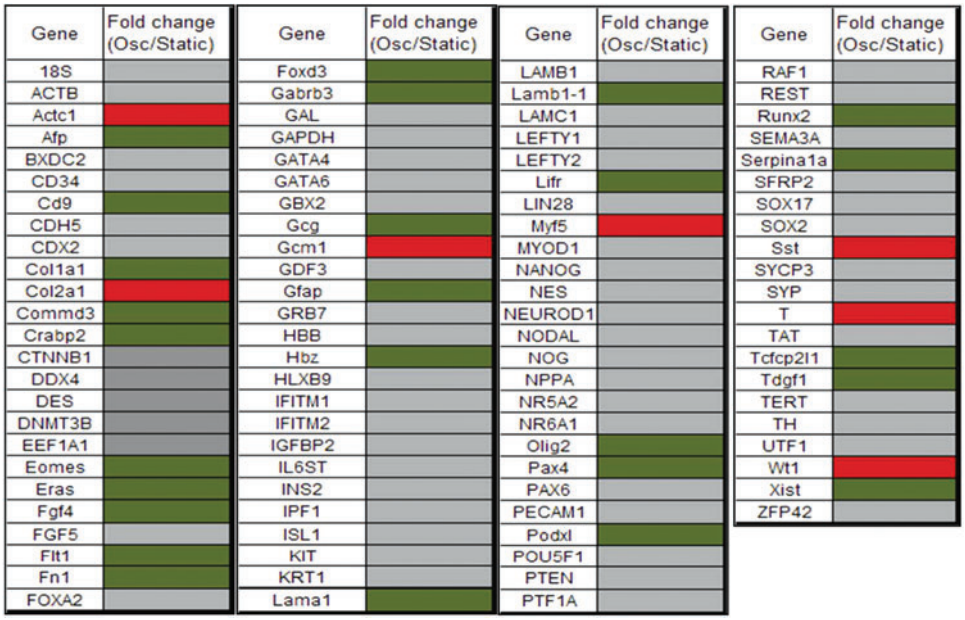
Examined genes are specifically associated with pluripotency and stemness or differentiation to ectoderm, mesoderm, or endoderm. The following list presents the 96 analyzed gene, where upregulated (red) or downregulated (green) genes were marked when the change was observed in more than 20% of at least three of four experiments.



Gene	Fold change (Osc/Static)
18S	
ACTB	
Actc1	
Afp	
BXDC2	
CD34	
Cd9	
CDH5	
CDX2	
Col1a1	
Col2a1	
Commd3	
Crabp2	
CTNNB1	
DDX4	
DES	
DNMT3B	
EEF1A1	
Eomes	
Eras	
Fgf4	
FGF5	
Flt1	
Fn1	
FOXA2	

Gene	Fold change (Osc/Static)
Foxd3	
Gabrb3	
GAL	
GAPDH	
GATA4	
GATA6	
GBX2	
Gcg	
Gcm1	
GDF3	
Gfap	
GRB7	
HBB	
Hbz	
HLXB9	
IFITM1	
IFITM2	
IGFBP2	
IL6ST	
INS2	
IPF1	
ISL1	
KIT	
KRT1	
Lama1	

Gene	Fold change (Osc/Static)
LAMB1	
Lamb1-1	
LAMC1	
LEFTY1	
LEFTY2	
Lifr	
LIN28	
Myf5	
MYOD1	
NANOG	
NES	
NEUROD1	
NODAL	
NOG	
NPPA	
NR5A2	
NR6A1	
Olig2	
Pax4	
PAX6	
PECAM1	
Podxl	
POU5F1	
PTEN	
PTF1A	

Gene	Fold change (Osc/Static)
RAF1	
REST	
Runx2	
SEMA3A	
Serpina1a	
SFRP2	
SOX17	
SOX2	
Sst	
SYCP3	
SYP	
T	
TAT	
Tcfcp2l1	
Tdgf1	
TERT	
TH	
UTF1	
Wt1	
Xist	
ZFP42	

SUPPLEMENTARY FIG. S3. TaqMan list of genes.

The following differentiation-associated genes were upregulated, and we elaborate their path in early differentiation of mESCs.

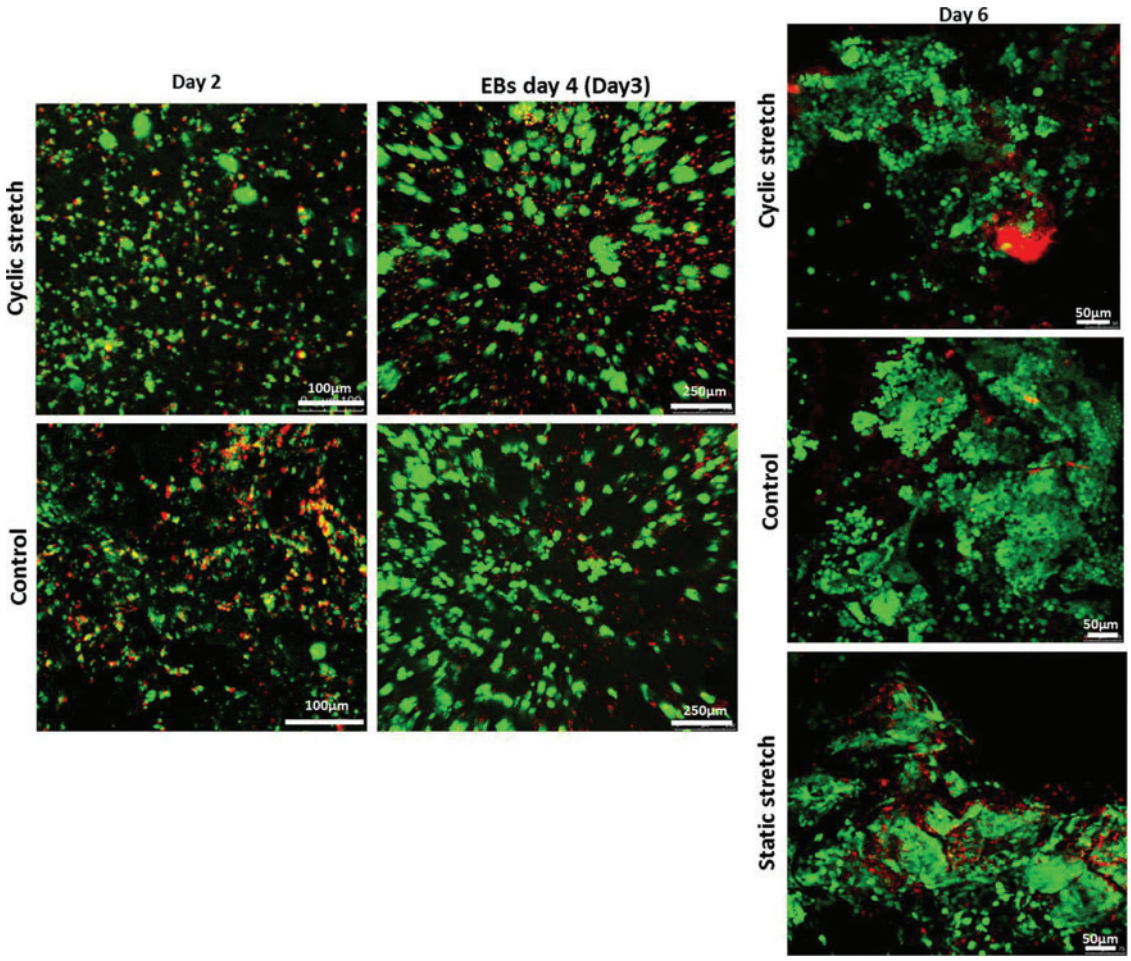
Actc1: related to early cardiac differentiation[1]
Col2a1: related to chondrogenesis[2]
Gcm1: related to trophectodermal differentiation[3]
Myf5: related to myogenic mesodermal[4]
Sst: related to endocrine cell[5]
Wt1: key regulator of mesenchyme to epithelial balance in the development of certain mesodermal organs and responsible for mesenchyme maintenance[6]

In the article (Fig. 5) we present the gene enrichment map correlating the changes in the examined genes with biological processes.

Viability of Cells Under Stretch Forces

To assess cell viability in the 3D scaffolds, constructs were loaded with calcein acetoxymethyl ester (calcein AM; 1 μM), which stains live cells, and with ethidium homodimer-1 (4 μM) (Sigma-Aldrich, St. Louis, MO), which stains dead cells, for 30 min at 37°C. Scaffolds were visualized using a confocal microscope. Viability quantification was performed using MATLAB (The Mathworks, Natick, MA). The total number of live (labeled in green) and dead (labeled in red) cells was quantified as the number of green/red pixels in the image and viability was determined as number of live cells divided by the total number of cells.

The following figure presents fluorescence images of scaffolds with cells.



SUPPLEMENTARY FIG. S4. Viability test for mESCs under stretch forces: green, live cells; red, dead cells.

Note that in the EB state, the cells were not entirely separated to single cells; therefore, in some cases, green dots present groups of cells.

Quantification of the images demonstrated high percentages (>80%) of viability under all conditions, as summarized in the following table.

	Day 2	*EBs day 4*	*Day 6*
Cyclic stretch	83.33	79.41 ± 1.83	90.71 ± 5.29
Control	81.6 ± 3.62	91.29 ± 0.85	98.68 ± 0.42

EBs, embryoid bodies.

References

1. Takeuchi, J.K., and Bruneau, B.G. Directed transdifferentiation of mouse mesoderm to heart tissue by defined factors. Nature **459,** 708, 2009.
2. Waese, E.Y., and Stanford, W.L. One-step generation of murine embryonic stem cell-derived mesoderm progenitors and chondrocytes in a serum-free monolayer differentiation system. Stem Cell Res **6,** 34, 2011.
3. Matin, M.M., Walsh, J.R., Gokhale, P.J., Draper, J.S., Bahrami, A.R., Morton, I., Moore, H.D., and Andrews, P.W. Specific knockdown of Oct4 and beta2-microglobulin expression by RNA interference in human embryonic stem cells and embryonic carcinoma cells. Stem Cells **22,** 659, 2004.
4. Sakurai, H., Sakaguchi, Y., Shoji, E., Nishino, T., Maki, I., Sakai, H., Hanaoka, K., Kakizuka, A., and Sehara-Fujisawa, A. *In vitro* modeling of paraxial mesodermal progenitors derived from induced pluripotent stem cells. PLoS One **7,** e47078, 2012.
5. Takeuchi, H., Nakatsuji, N., and Suemori, H. Endodermal differentiation of human pluripotent stem cells to insulin-producing cells in 3D culture. Sci Rep **4,** 4488, 2014.
6. Chau, Y.Y., and Hastie, N.D. The role of Wt1 in regulating mesenchyme in cancer, development, and tissue homeostasis. Trends Genet **28,** 515, 2012.