

**Mesenchymal morphogenesis of embryonic stem cells dynamically
modulates the biophysical microtissue niche**

Melissa A. Kinney¹, Rabbia Saeed¹, and Todd C. McDevitt^{*1,2}

¹The Wallace H. Coulter Department of Biomedical Engineering
Georgia Institute of Technology/Emory University, Atlanta, GA, USA

²The Parker H. Petit Institute for Bioengineering and Bioscience,
Georgia Institute of Technology, Atlanta, GA, USA

Address for Correspondence:

Todd C. McDevitt, Ph.D.

313 Ferst Drive, Suite 2102

Atlanta GA, 30332-0532

Phone: 404-385-6647

Fax: 404-894-4243

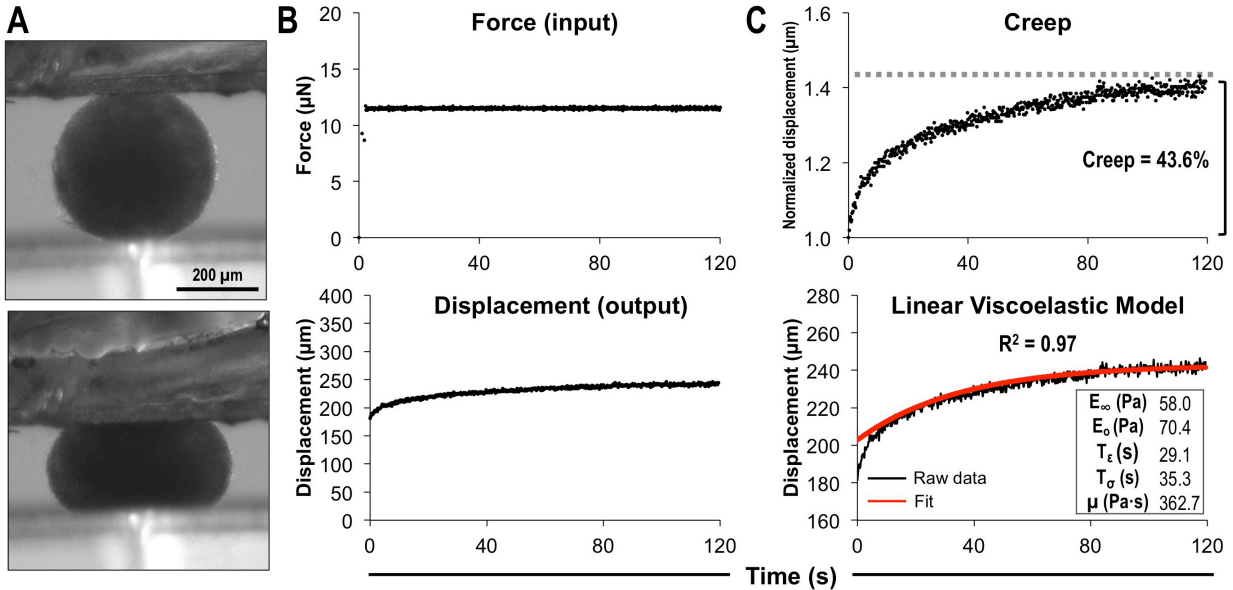
Email: todd.mcdevitt@bme.gatech.edu

Supplemental Methods

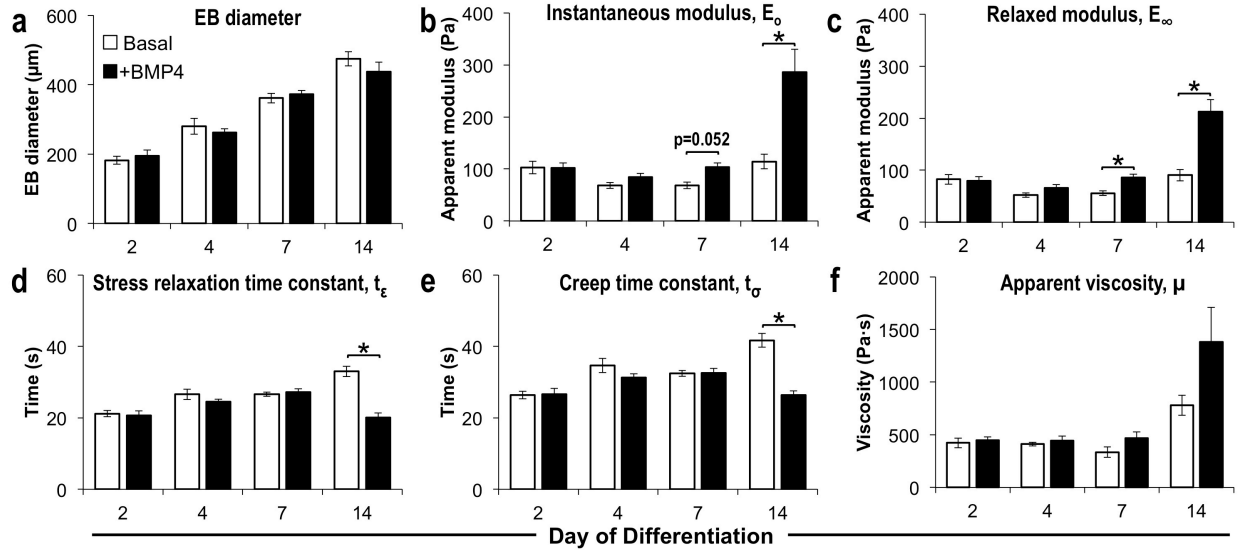
Immunostaining

For whole mount immunostaining, formalin fixed EBs were blocked and permeabilized in 2% BSA/0.1% Tween-20, containing 1-1.5% Triton X-100 for 30 minutes at 4°C with rotation, re-fixed in formalin for 15 minutes and blocked for an additional 3 hours. EBs were then stained with Alexa Fluor 546 phalloidin (1:40, Molecular Probes), counterstained with Hoechst (10 $\mu\text{g}/\text{mL}$) and imaged using a Zeiss LSM 710 Confocal Microscope.

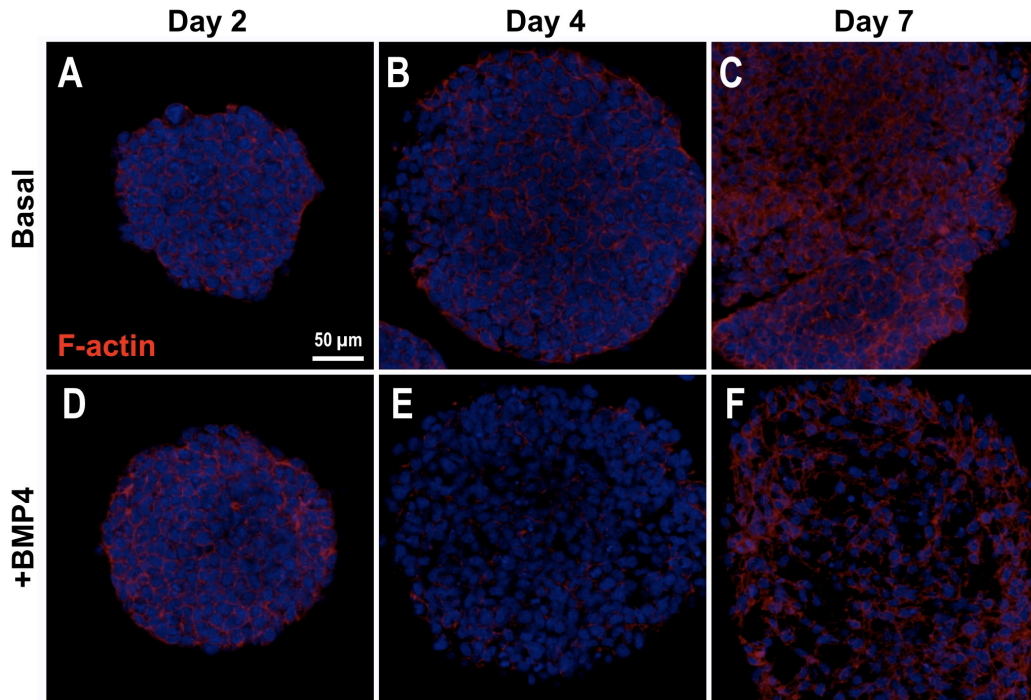
Supplemental Figures



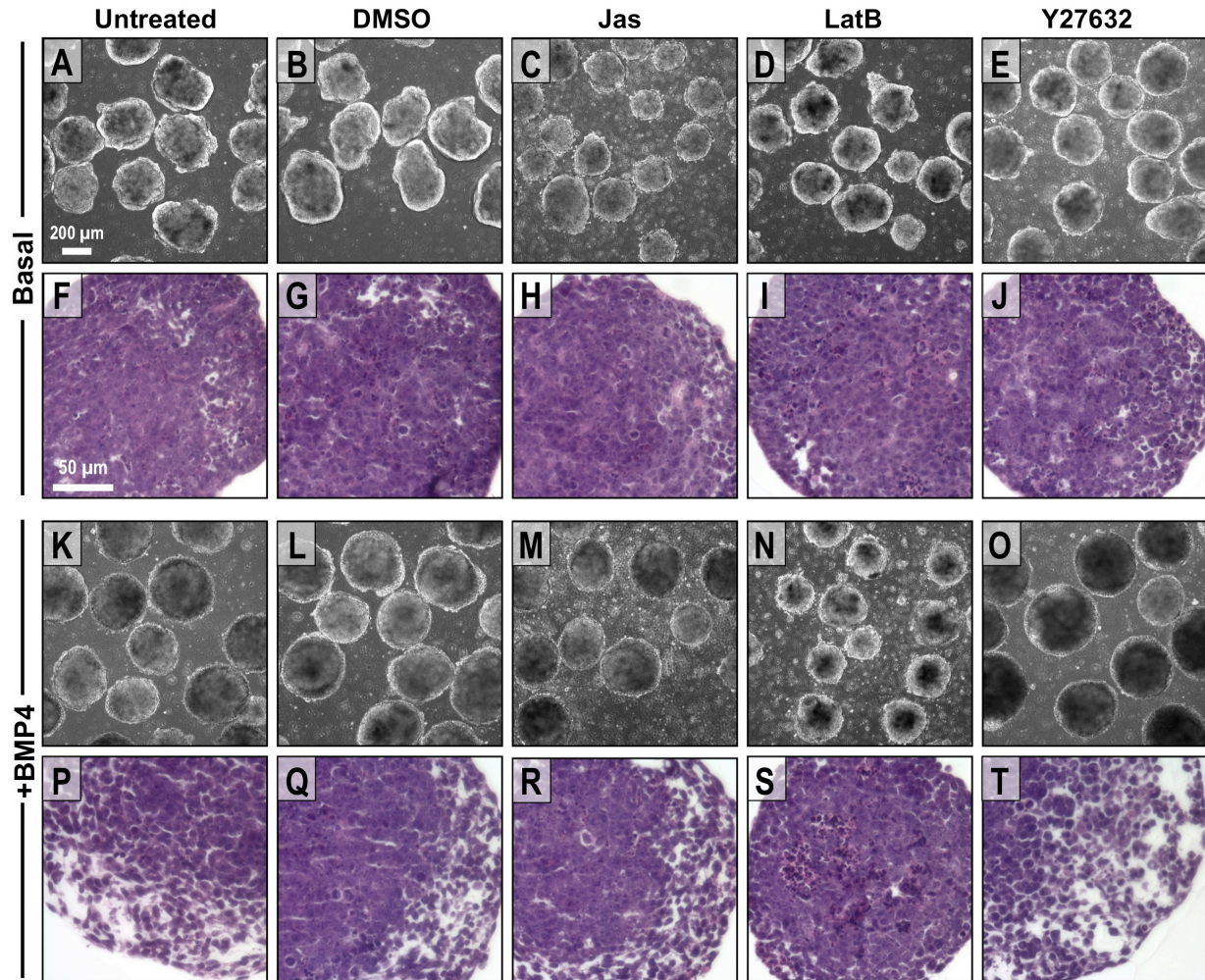
Supplemental Figure 1. Mechanical testing methods. (A) Mechanical testing of EBs was accomplished through parallel plate compression, in which (B) the displacement was measured over time under constant force. (C) The resultant creep curves enable calculation of viscoelastic properties, including characteristic creep displacement, as well as parameters determined from the fit to a linear viscoelastic model. Scale bar = 200 μm .



Supplemental Figure 2. Mechanical characterization. (A) Although EB diameter was not significantly modulated by culture environment, treatment with BMP4 resulted in increased (B) instantaneous and (C) relaxed moduli after 7 and 14 days of differentiation, as well as decreased (D) stress relaxation and (E) creep time constant. (F) The apparent viscosity increased over time, but was not modulated by culture condition. $n=6$ EBs; $* = p < 0.05$.



Supplemental Figure 3. F-actin localization within EBs during differentiation. EBs displayed largely cortical F-actin structures, which were dynamically remodeled during the course of differentiation after 2 (A&D), 4 (B&E), and 7 (C&F) days of differentiation in basal, serum free cultures (A-C) or upon soluble treatment with BMP4 (D-F). Scale bar = 50 μm .



Supplemental Figure 4. Morphology of EBs cultured with cytoskeletal agonists and antagonists. Phase images (A-E, K-O) and H&E staining (F-J, P-T) of EBs cultured in basal, serum free media (A-J) or upon supplementation with BMP4 (K-T) exhibit changes largely consistent with the soluble growth factor treatment, with few subtle changes due to cytoskeletal perturbation with Jas (C, H, M & R), LatB (D, I, N & S), and Y27632 (E, J, O & T). Scale bar (A) = 200 μm, (F) = 50 μm.

Table 1. PCR array genes and classifications

| ID | Gene name | Primary classification |
|---------------|---|-------------------------------|
| Nanog | Nanog | Pluripotency |
| Oct-4 | Octamer binding transcription factor-4 (POU5F1) | Pluripotency |
| Sox2 | sex determining region Y-box 2 | Pluripotency |
| Foxa2 | forkhead box protein A2 (HNF-3 β) | Endoderm |
| Gata4 | GATA binding protein 4 | Mes/endoderm |
| Gata2 | GATA binding protein 2 | Mesoderm |
| Afp | Alpha fetoprotein | Endoderm |
| Nes | Nestin | Ectoderm |
| Sox17 | sex determining region Y-box 17 | Ectoderm |
| T | Brachyury-T | Mesoderm |
| Pecam1 | Platelet endothelial cell adhesion molecule | Mesoderm / endothelial |
| Cdh5 | Vascular endothelial cadherin (VE-cadherin) | Mesoderm / endothelial |
| Des | Desmin | Mesoderm / cardiac |
| Pax6 | Paired box gene 6 | Ectoderm |
| Runx2 | Runt-related transcription factor 2 | Mesoderm / osteogenic |
| Fgf5 | Fibroblast growth factor 5 | Growth factor |
| Bmp4 | Bone morphogenetic protein 4 | Growth factor |
| Kdr | Vascular endothelial growth factor receptor 2 | Mesoderm / endothelial |
| Nkx2.5 | homeobox protein Nkx-2.5 | Mesoderm / cardiac |
| Cd34 | Cluster of differentiation 34 | Mesoderm / hematopoietic |
| Sox4 | sex determining region Y-box 4 | Mesoderm / hematopoietic |
| Vegfa | Vascular endothelial growth factor A | Mesoderm / endothelial |
| Flt1 | Vascular endothelial growth factor receptor 1 | Mesoderm / endothelial |
| Igf2 | Insulin-like growth factor 2 | Growth factor |
| Hba-x | hemoglobin X, alpha-like embryonic chain | Mesoderm / hematopoietic |
| Hbb-y | hemoglobin Y, beta-like embryonic chain | Mesoderm / hematopoietic |
| 18s | Ribosomal protein S18 | Housekeeping |
| Actb | Beta actin | Housekeeping |
| Gapdh | Glyceraldehyde 3-phosphate dehydrogenase | Housekeeping |