

Supplemental Figures

Combinatorial extracellular matrix cues with mechanical strain induce differential effects on myogenesis *in vitro*

Alex H.P. Chan^{1,2,3#}, Ishita Jain^{1,2,3#}, Beu P. Oropeza^{1,2,3}, Tony Zhou^{1,2},
Brandon Nelsen⁴, Nicholas A. Geisse⁴, Ngan F. Huang^{1,2,3,5 *}

¹ Stanford Cardiovascular Institute, Stanford, CA

² Department of Cardiothoracic Surgery, Stanford University, Stanford, CA

³ Center for Tissue Regeneration, Repair and Restoration; Veterans Affairs Palo Alto Health Care System, Palo Alto, CA

⁴ Curi Bio, Seattle WA, USA

⁵ Department of Chemical Engineering, Stanford University, Stanford, CA 94305, USA

Contributed equally

* To whom correspondence should be addressed:

Dr. Ngan F. Huang, PhD

Associate Professor

Department of Cardiothoracic Surgery

Stanford University

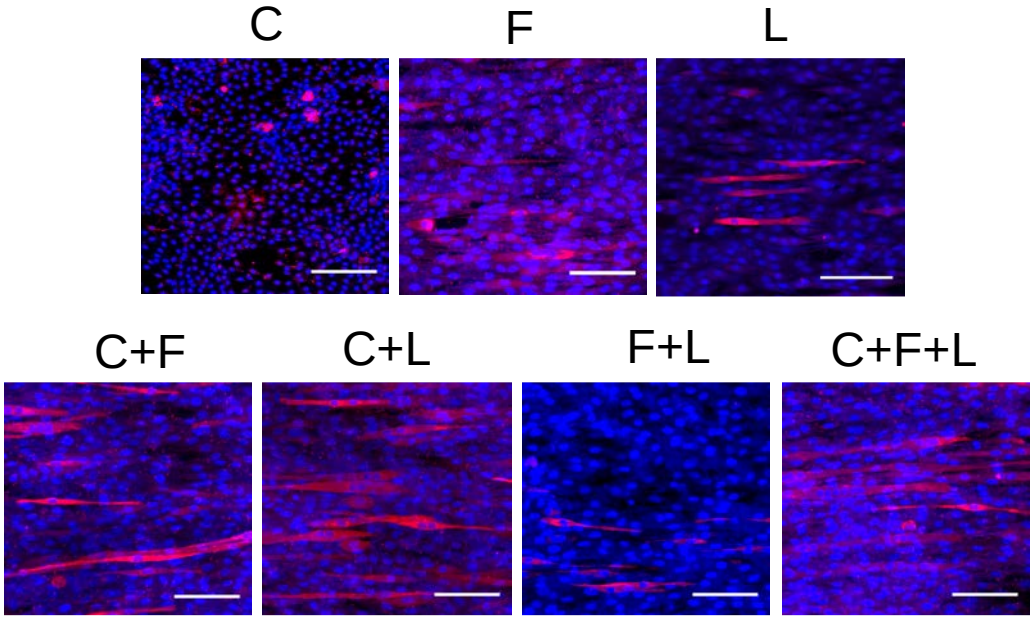
300 Pasteur Drive, MC 5407

Stanford, CA 94305-5407

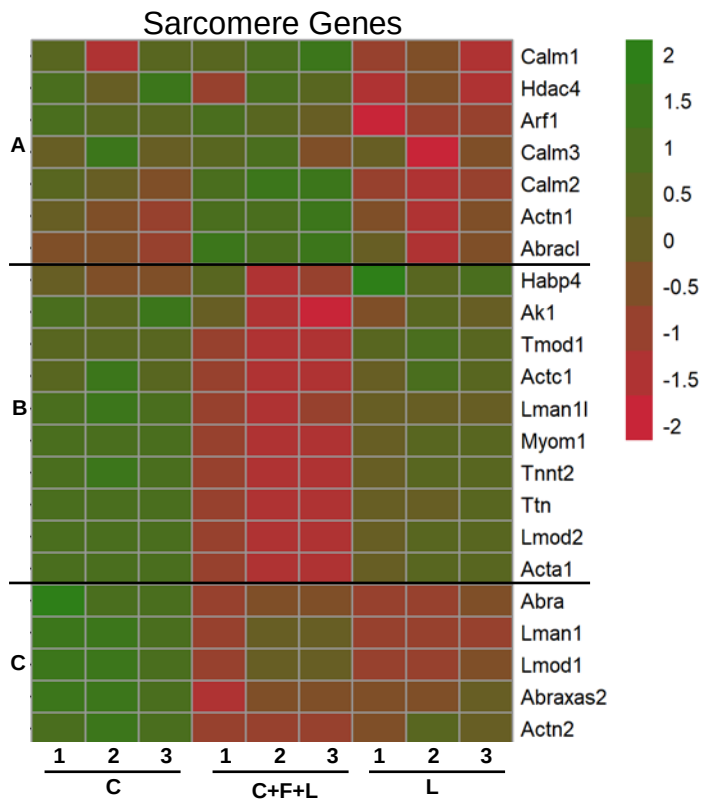
Tel: 650-849-0559

Email: ngantina@stanford.edu

ORCID Identifier: 0000-0003-2298-6790



Supplementary Figure 1. Representative fluorescent images of myoblasts on combinatorial ECMs under static conditions: C2C12 myoblasts cultured on collagen I (C), fibronectin (F), laminin (L), collagen I + fibronectin (C+F), collagen I + laminin (C+L), fibronectin + laminin (F+L), and collagen I + fibronectin + laminin (C+F+L). Blue: Hoechst 33342 nuclear dye; Red: myosin heavy chain. Scale bar: 100 μ m.



Supplementary Figure 2. Quantification of sarcomere-related gene expression based on RNA Sequencing. Data is shown for collagen I (C), laminin (L), and collagen I + fibronectin + laminin (C+F+L) extracellular matrices under static conditions.

Constant

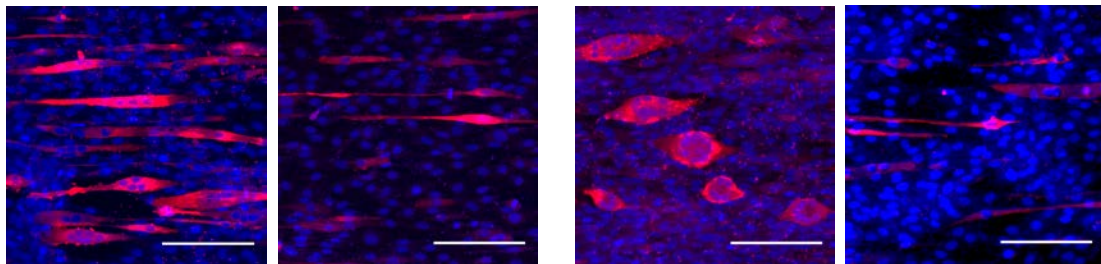
High

Low

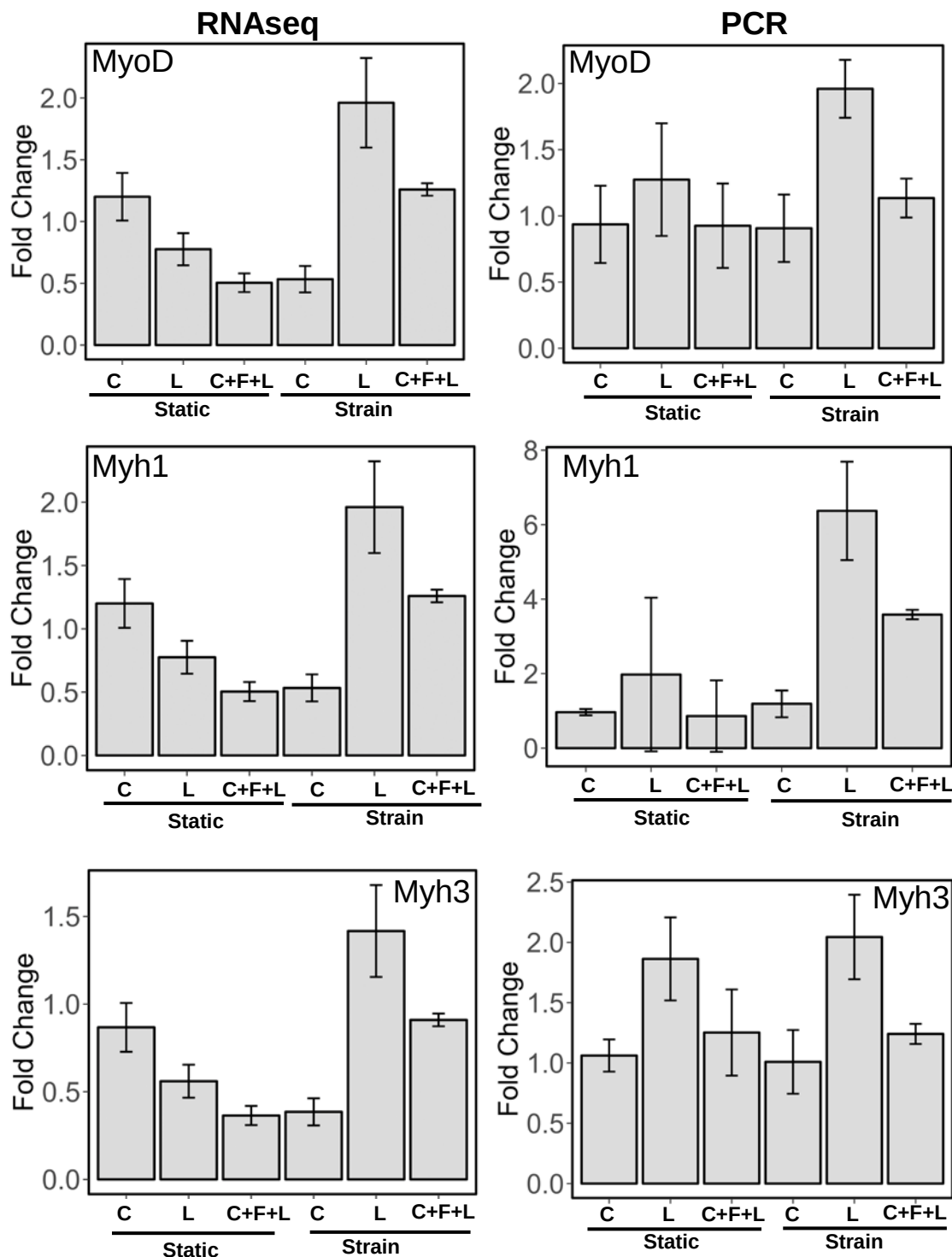
Intermediate

High

Low



Supplementary Figure 3: Representative fluorescent images of C2C12 myoblasts cultured on laminin under different stretch regimes (constant stretch: high or low; intermediate stretch: high or low). Blue: Hoechst 33342 nuclear dye; Red: myosin heavy chain. Scale bar: 100 μm .



Supplementary Figure 4. Comparison of gene expression data from RNAseq to quantitative PCR. Fold change gene expression with respect to Collagen I on static calculated for both RNAseq and qPCR. For qPCR, the data was normalized to GAPDH housekeeping gene (n = 2-3). Data is shown for collagen I (C), laminin (L), and collagen I + fibronectin + laminin (C+F+L).