**Supplemental Movie 1: Isolated Cardiomyocyte Beating on Soft Matrix**

Depicted here is the principal strain field (left) created by the cell (right) as it deforms the soft gel with a basal level of traction forces. As the cardiomyocyte contracts the gel, however, strain rapidly increases as a result of increased traction forces, which also decay rapidly again. The magnitude of the strain field is determined from bead displacements and uses the same strain scale as in Figure 1.

**Supplemental Movie 2: Isolated Cardiomyocyte Beating on Stiff Matrix**

Shown here is the principal strain field (left) created by the cell (right) as it attempts to deform the stiff gel. Traction forces are small, but non-zero. As the cardiomyocyte contracts, the resulting traction forces do not greatly change, although the cell significantly contracts. Strain magnitude uses the same scale as in Figure 1.

**Table S1: Summary of In Situ Cysteine Labeling in the Non-muscle Myosin IIB Head and Lever Arm for Cardiomyocytes on Stiff Matrices**

| Cys | Location | Detected | % Labeled | Surface Accessible? |
|---|---|---|---|---|
| 95 | Head Domain | Yes | 25% | Yes |
| 122 | Head Domain | No | - | No |
| 164 | Head Domain | No | - | Partial[1] |
| **176** | **Head Domain/ATP[4]** | **Yes** | **0%** | **No** |
| 382 | Head Domain | Yes | 0% | Yes |
| 475 | Head Domain/ABD[5] | No | - | Partial[2] |
| 518 | Head Domain/ABD | No | - | Partial[2] |
| 539 | Head Domain/ABD | No | - | No |
| 576 | Head Domain | Yes | 0% | Yes |
| **678** | **Head Domain/ATP** | **Yes** | **0%** | **No** |
| **701**[6] | **Head Domain/ATP** | **Yes** | **25%** | **No** |
| 711 | Head Domain | No | - | No |
| 747 | Head Domain | No | - | Partial[1] |
| 797 | Lever Arm | No | - | Partial[3] |
| 800 | Lever Arm | No | - | Partial[3] |
| 823 | Lever Arm | Yes | 0% | Partial[3] |

Sites labeled in bold indicate those that were detected by mass spectrometry but are not surface accessible according to protein structure.

[1]Partially accessible on an exterior α-helix or β-sheet but on the interior portion of that secondary structure.

[2]Partially accessible because often bound to actin

[3]Partially accessible because normally bound to myosin light chains

[4]ATP: ATP binding cleft

[5]ABD: Actin-binding domain

[6]The identical peptide fragment is found also in human cardiac myosin heavy chain.

**Figure S1: AFM Measurement of Myocardial Elasticity**

(**A**) Brightfield image of freshly isolated day 4 myocardium. Sample is then mounted on a AFM stage as depicted schematically with the apical surface exposed. An AFM tip with r ~ 50 nm and experimentally determined spring constant, $k_{sp}$, is then indented into the myocardium to yield tip deflection, *d*, versus tip position, *z* (**B**). Elasticity was determined by fitting a hertz cone model from the contact to the maximal indentation points, with the former determined by a range of analysis method from $z_1$, $d_1$ to $z_2$, $d_2$.

**Figure S2: Cardiomyocytes at High Density also show Beating depends on Matrix Elasticity** Purified cardiomyocytes were plated at high density on matrices of varied stiffness, and beating was observed in phase contrast. (**A**) Beat frequency is elasticity-dependent, but decreases in beat frequency are smaller compared to low density cultures. (**B**) The percentage of cells beating at high density was higher compared to low density cultures, though stiff matrices above *E** still induced loss of beating over time. Error bars are SD for >5 cells, 10 sec each in triplicate studies.

**Figure S3: Assay for Glutathione shows no difference between Cardiomyocytes on Soft and Stiff Matrices** (**A**) Representative chromatogram from Reverse Phase HPLC separation of hydrophilic glutathione (GSH; MW = 307 Da) derivatized with excess of hydrophobic mBBr (MW 271 Da). The two wavelengths were used to identify where the peptide absorbs (220 nm) and where mBBr alone has an absorption peak (396 nm). Free GSH (dotted line) elutes early (~2.5 min), whereas free mBBr elutes at 18 min. mBBr-GSH shows two peaks that absorb significantly at both 220 and 396 nm. (**B**) Total

levels of reacted GSH-mBBr product, determined by integrating the appropriate peaks, are similar for cardiomyocytes grown on soft or hard matrices for 24 h.

**Figure S4: Labeled Cysteine Sites in Structures of Filamin-like Actin Binding Domain and Myosin II head.** The sites were identified in Cys Shotgun labeling with mBBr of live cardiomyocytes on hard matrix. (**A**) The actin binding domain structure of human α-actinin-3 (1TJT), combined with sequence alignment to filamin A and B, identifies the location of C59 (in filamin A) at the end of helix-A near a conserved, actin-binding Asn. (**B**) The structure of the head of chicken smooth muscle myosin II (1BR2), combined with sequence alignment to human nonmuscle myosin IIB and cardiac myosin, identifies the location of the labeled and partially buried thiol (SH1) at one end of the canonical SH1-SH2 helix.

Figure S1

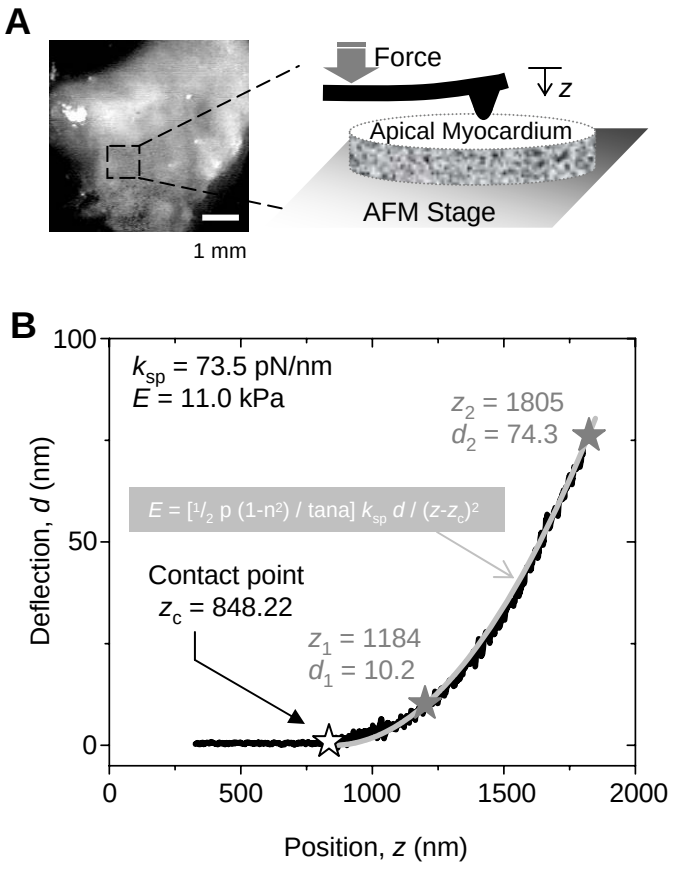



Figure S2

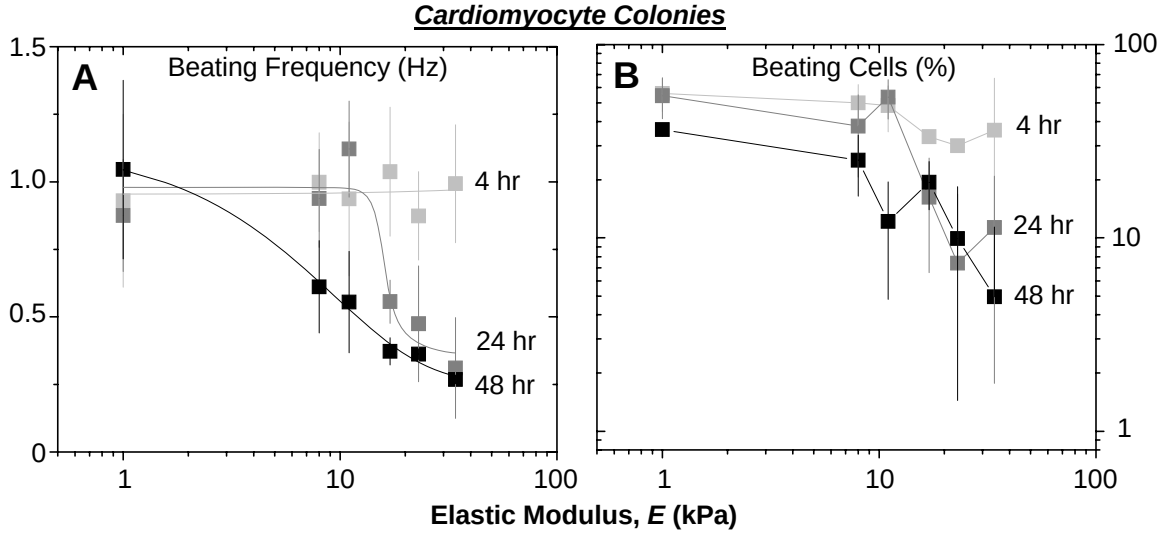
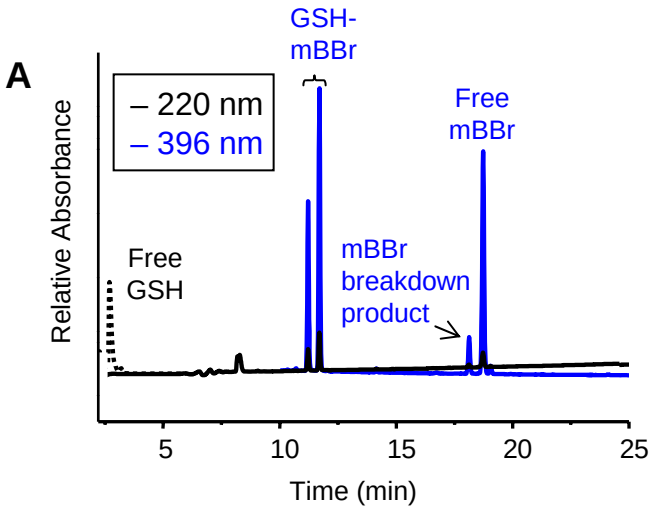


Figure S3




B

| $E_{matrix}$ | Total GSH-mBBR in Cardiomyocytes (relative Abs) |
|---|---|
| 1 kPa | **1010** |
| 34 kPa | **1023** |

Figure S4