**SUPPLEMENTARY**

# Tissue-engineered 3-dimensional (3D) microenvironment enhances the direct reprogramming of fibroblasts into cardiomyocytes by microRNAs.

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## Supplementary Figures

**Supplementary Figure 1. Design of the 3D tissue bundles.** (A) Fibroblasts were mixed in a fibrin/matrigel matrix and then cast into wells of PDMS molds and anchored to nylon frames. After one day of compaction, frames with bundles were removed for free-floating culture. (B) Representative live images of 2-week old bundles with fibroblasts transfected with either Neg miR or miR combo. (C) Quantification of bundle diameters (N=7). **** $P < 0.0001$.

**Supplementary Figure 2. miR combo reduces passive force in 3D cultures.** Passive force-length relationships in 3D tissue bundles made of fibroblasts transfected with negative control miR (negmiR) or miR combo and cultured for 14 days (N=3). * $P<0.05$ between negmiR and miR combo. Passive force-length of a neonatal rat ventricular myocytes (NRVMs) enriched population is provided by way of comparison.

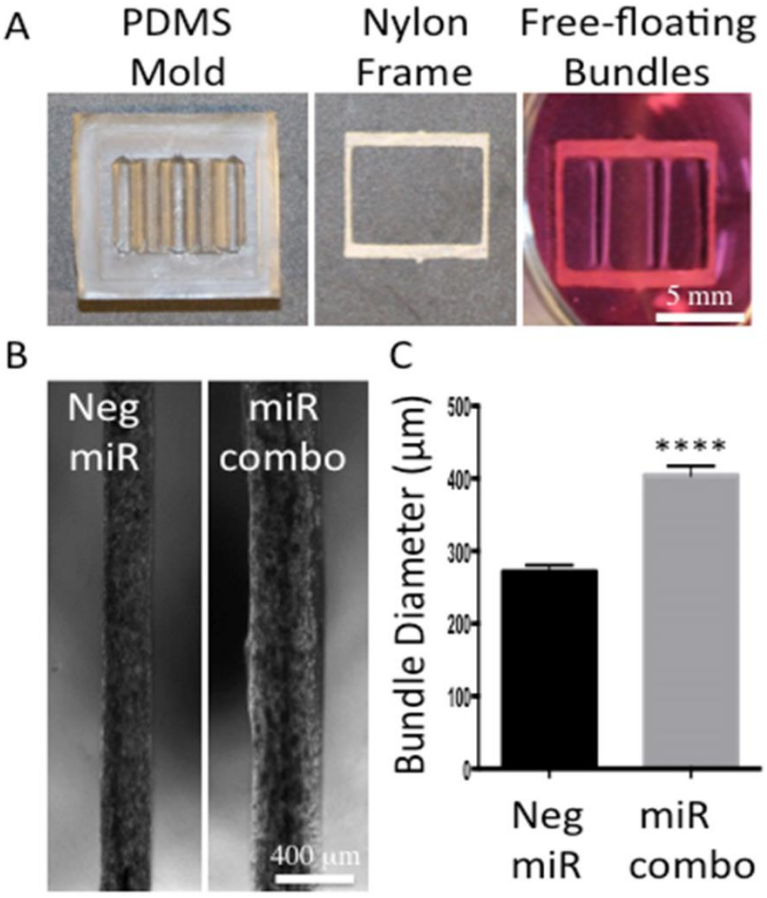
**Supplementary Figure 3. miR combo reprogramming in Fsp1+ fibroblasts.** (A) Representative immunostaining images of neonatal mouse cardiac fibroblasts derived from Fsp1Cre:tdTomato mice transfected with miR combo and cultured for 14 days in 2D or 3D environment. (B) Quantitative comparison of cTnT/TdTomato and SAA/TdTomato expressing

cells in 2D and 3D culture. * P<0.05, ** P<0.01. Comparisons made between 2D and 3D groups.

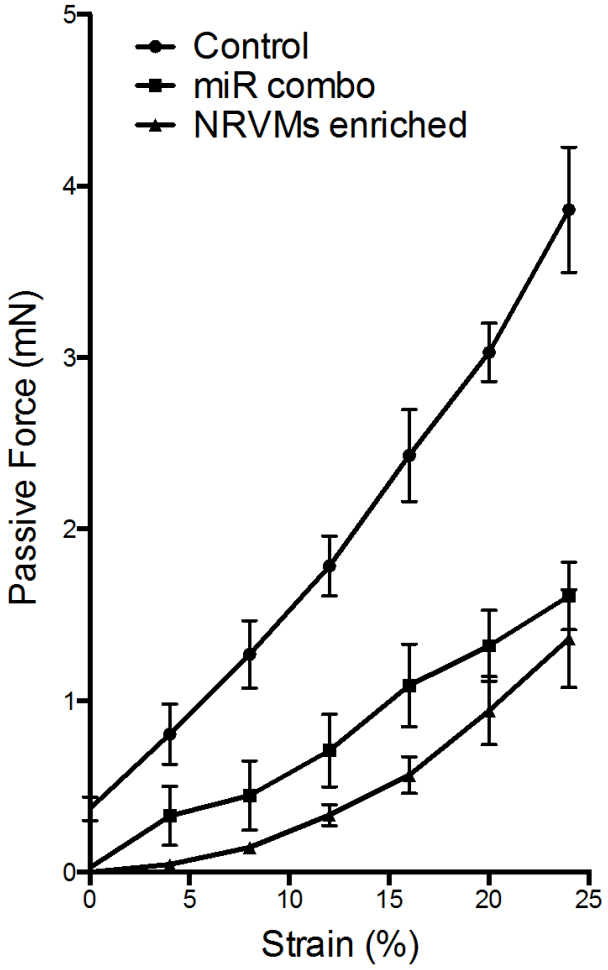
**Supplementary Figure 4**. **Gene expression in 3D vs. 2D culture environment.** Expressions of 84 genes important for cell-cell and cell-matrix interactions were assessed using a qPCR array (RT² Profiler™ PCR Array Mouse Extracellular Matrix & Adhesion Molecules, QIAGEN) in 14-day 2D and 3D cultures of miR combo transfected cardiac fibroblasts. Table and graph show the genes with significantly ($p<0.05$) upregulated expression in 3D compared to 2D environment.

**Supplementary Figure 5. MMP inhibition blocks the effect of the 3D environment upon cardiac reprogramming.** Neonatal cardiac fibroblasts were transfected with negative control miR (negmiR) or miR combo. Two days after transfection cells were re-plated either in regular culture dishes (2D) or encapsulated in a 3D hydrogel (3D). Cells were cultured for a further 14 days in the presence of vehicle or 5 uM BB94 (a broad spectrum MMP inhibitor). Cardiac gene expression was analyzed by qPCR. N=3, comparisons were not significant.

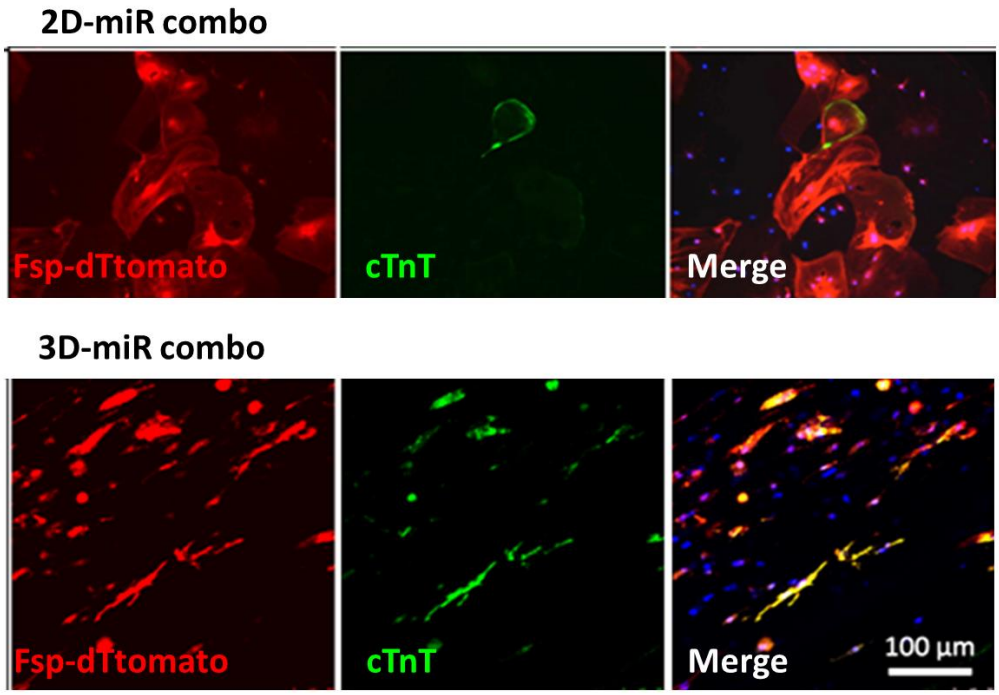
## Supplementary Figure 1

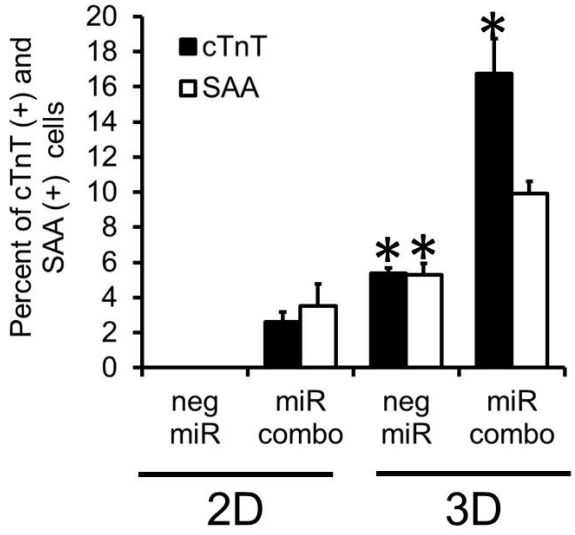


## Supplementary Figure 2

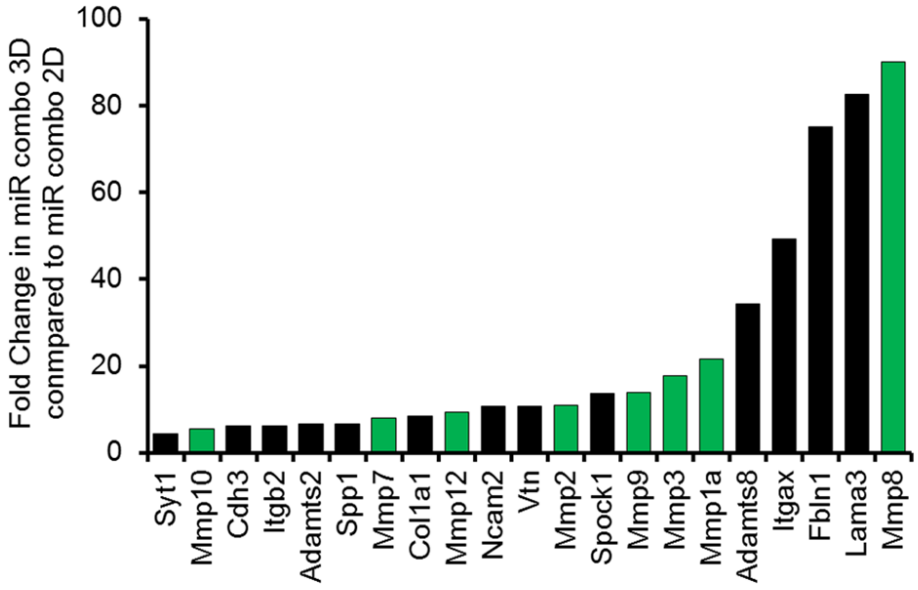


# Supplementary Figure 3



# Supplementary Figure 4




| Gene Name | Fold change miR combo 3D vs miR combo 2D |
|---|---|
| Syt1 | 4.3517 |
| **Mmp10** | **5.5094** |
| Cdh3 | 6.0892 |
| Itgb2 | 6.2255 |
| Adamts2 | 6.609 |
| Spp1 | 6.6848 |
| **Mmp7** | 7.9427 |
| Col1a1 | 8.4255 |
| **Mmp12** | **9.2822** |
| Ncam2 | 10.5986 |
| Vtn | 10.5986 |
| **Mmp2** | **10.9829** |
| Spock1 | 13.7507 |
| **Mmp9** | **13.9811** |
| **Mmp3** | **17.7361** |
| **Mmp1a** | **21.5201** |
| Adamts8 | 34.1876 |
| Itgax | 49.3464 |
| Fbln1 | 74.9954 |
| Lama3 | 82.4821 |
| **Mmp8** | **90.0245** |

# Supplementary Figure 5