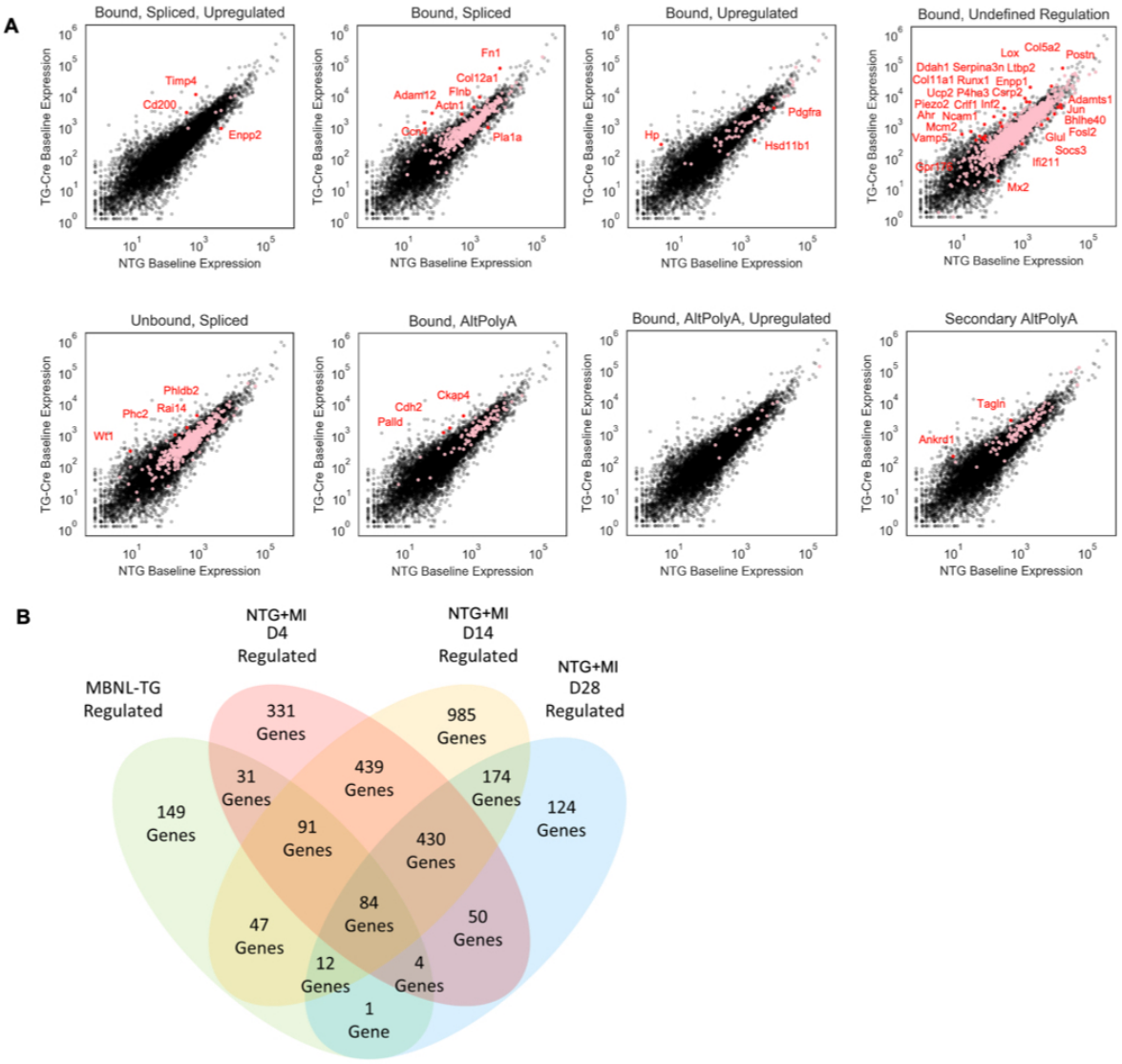
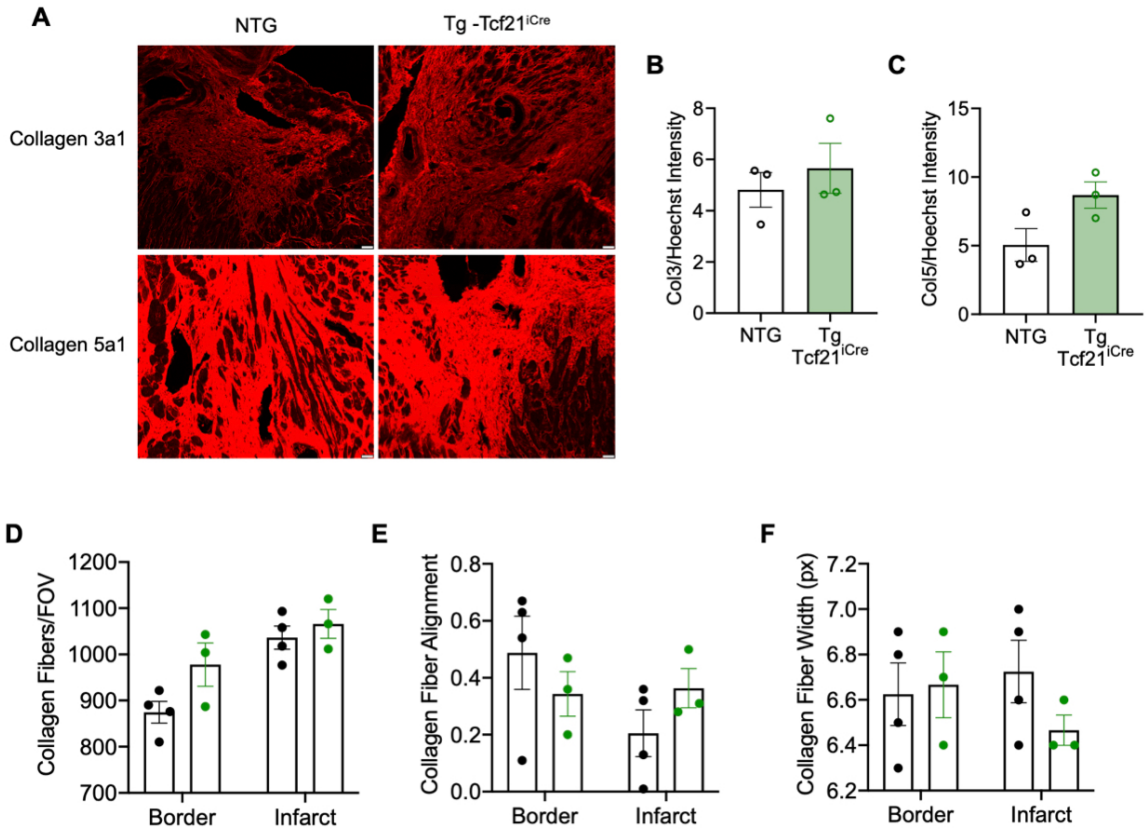
Supplemental Figure 1



Supplemental Figure 1: MBNL1 regulated transcripts identified in RNAseq.
(A) Scatterplot of differentially expressed genes between MEFSK4 sorted cardiac fibroblasts from MBNL1 Tg-Tcf21^{iCre} versus NTG-Tcf21^{iCre} with transcripts identified in previous RIPseq identified in red. **(B)** Venn diagram showing similarly regulated genes between sham operated MBNL1 Tg-Tcf21^{iCre} cardiac fibroblasts and NTG cardiac fibroblasts throughout the time course of MI relative to baseline NTG fibroblasts. Related to **Figure 2**

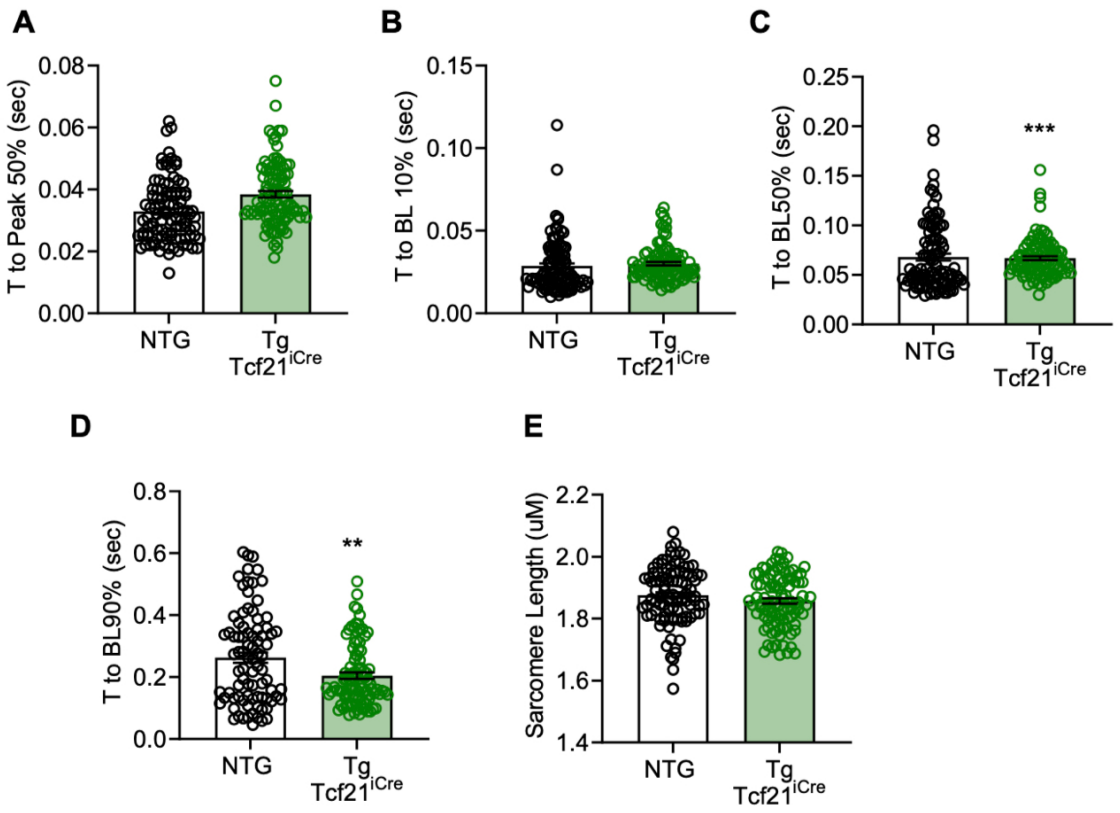
Supplemental Figure 2



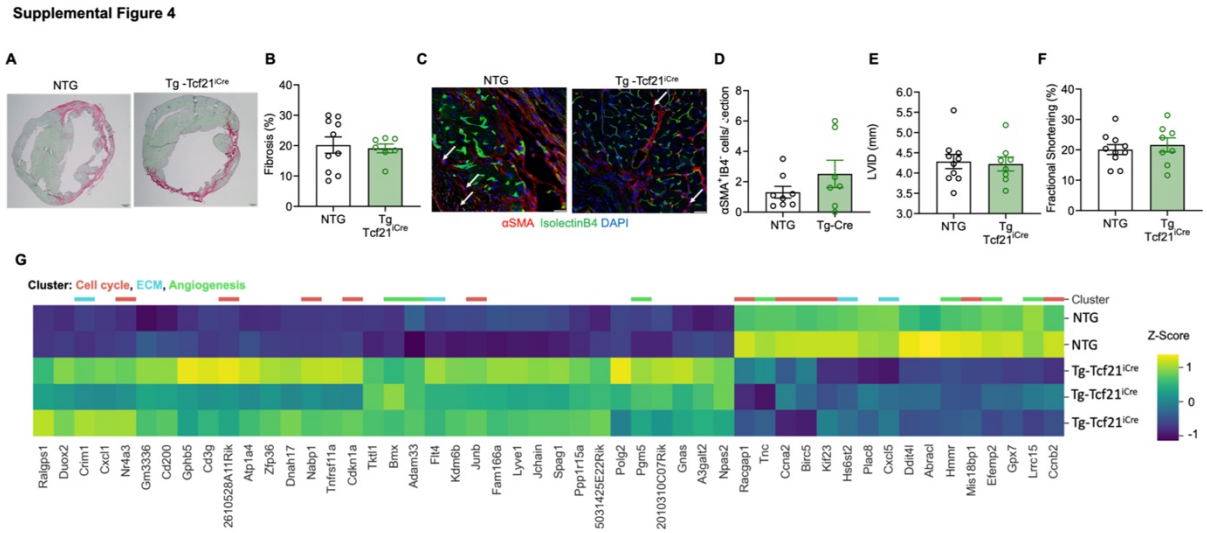
Supplemental Figure 2: Collagen composition and alignment in MBNL1 overexpression mice 30 days following MI.

(A) Histological images of Collagen 3a1 (top) and Collagen 5a1 (bottom). Quantification of **(B)** Collagen 3a1 (p=0.5150) and **(C)** Collagen 5a1 (p=0.0769) stain intensity normalized to Hoechst signal in MBNL1 Tg-Tcf21^{iCre} (n=3) and NTG-Tcf21^{iCre} (n=3) mice 14 days post MI. Dots represent biological replicates, bars represent mean±SEM, unpaired t-tests. Quantification of **(D)** the number of collagen fibers per field of view (Border p=0.0841, Infarct p=0.4887), **(E)** collagen fiber alignment (Border p=0.4244, Infarct p=0.2199) and **(F)** collagen fiber width (Border p=0.8457, Infarct p=0.1939) from second harmonic generation of decellularized hearts 30 days post MI. Dots represent biological replicates, MBNL1 Tg-Tcf21^{iCre} (n=3) and NTG-Tcf21^{iCre} (n=4), bars represent mean±SEM, unpaired t-tests. Related to **Figure 3**.

Supplemental Figure 3

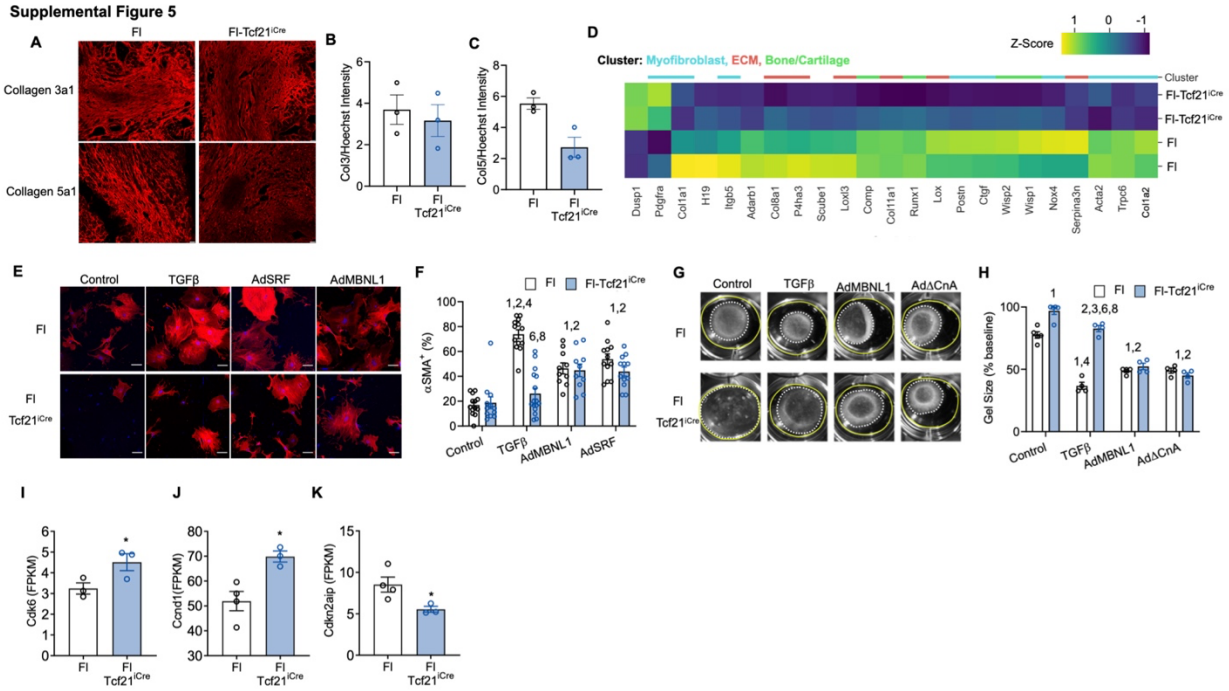


Supplemental Figure 3: Fibroblast specific overexpression of MBNL1 alters myocyte function following MI.
(A) Time to 50% of peak contraction. Relaxation time from peak contraction to **(B)** 10% and **(C)** 50%, and **(D)** 90% of baseline. **(E)** Diastolic sarcomere length. Dots represent individual myocytes (30/mouse), MBNL1 Tg-Tcf21^{iCre} (n=3 mice) and NTG-Tcf21^{iCre} (n=3 mice), bars represent mean±SEM, unpaired t-tests,****p<0.0001, ***p<0.001, **p<0.01. Related to **Figure 3**.

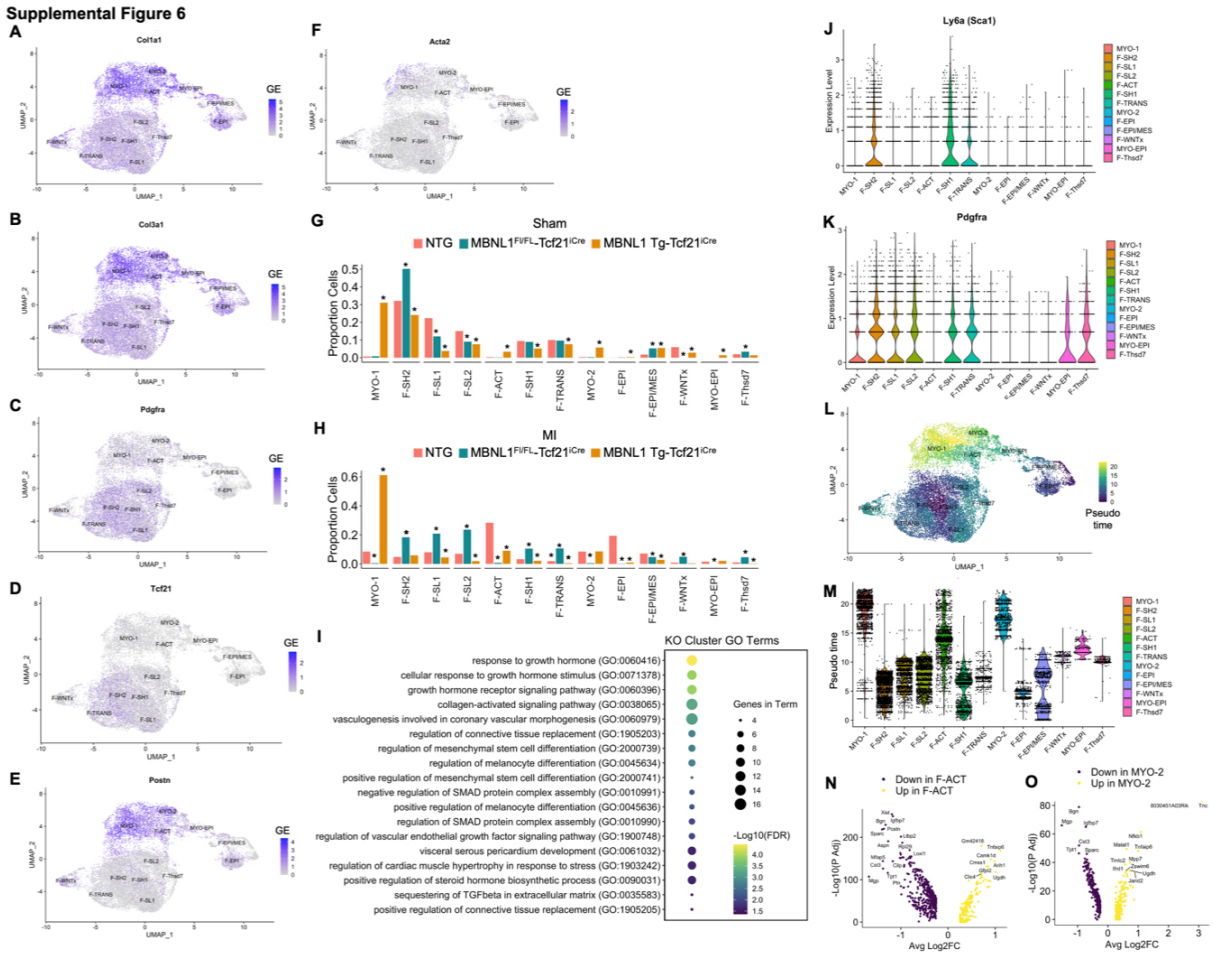


Supplemental Figure 4: Overexpression of MBNL1 in cardiac fibroblasts promotes a myofibroblast transcriptional state.

(A) Images and **(B)** quantification of Sirius Red/Fast Green-stained myocardial sections. Dots represent biological replicates, bars represent mean±SEM, unpaired t-tests, p=0.7575 from MBNL1 Tg-Tcf21^{iCre} (n=7) and NTG-Tcf21^{iCre} (n=10) mice 3 months post MI. **(C)** Immunofluorescent imaging and **(D)** quantification of myofibroblasts in the border zone of myocardial sections from MBNL1 Tg-Tcf21^{iCre} (n=7) and NTG-Tcf21^{iCre} (n=8) mice 3 months following MI. Myofibroblasts are αSMA (red) positive and negative for the endothelial marker isolectinB4 (IB4, green). Nuclei are stained blue. Arrows show αSMA^{+}IB4^{-} cells. Scalebar=25μm p=0.2207. Echocardiographic analysis of left ventricle **(E)** diastolic chamber diameter (p=0.8109) and **(F)** fractional shortening (p=0.5840), 3 months post MI in MBNL1 Tg-Tcf21^{iCre} (n=8) and NTG-Tcf21^{iCre} (n=10) mice. Dots represent biological replicates, bars represent mean±SEM, unpaired t-tests for statistical comparison. **(G)** Heatmap of differentially expressed genes in MBNL Tg-Tcf21^{iCre} versus NTG-Tcf21^{iCre} cardiac fibroblasts 4 days post MI. Colored bars represent functionally clustered genes: Extracellular matrix (ECM) = blue, Cell cycle = red, and Angiogenesis = green. Related to **Figure 3**.

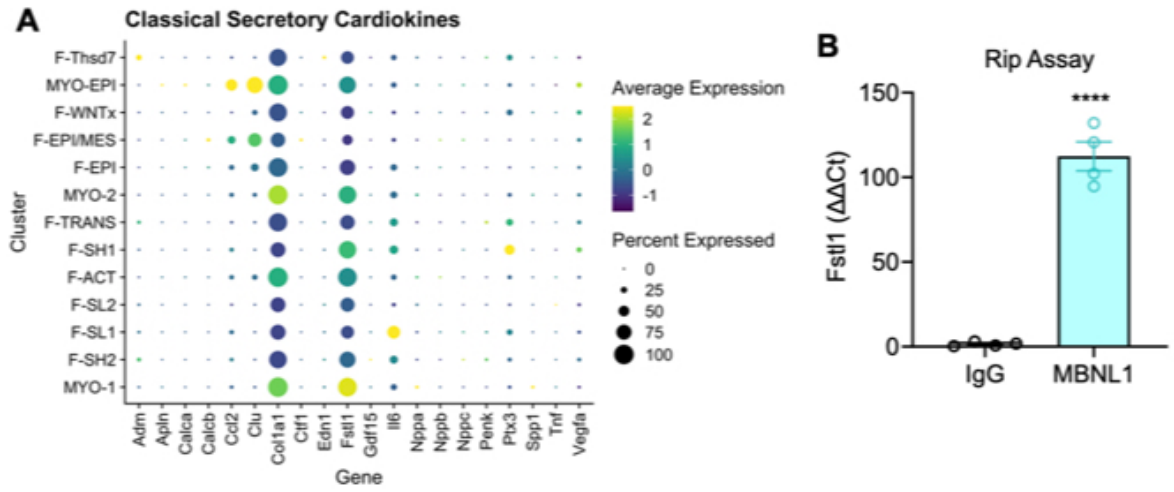


Supplemental Figure 5: MBNL1 is required for myofibroblast phenotype and function.
(A) Images of Collagen 3a1 (top) and Collagen 5a1 (bottom). Quantification of **(B)** Collagen 3a1 ($p=0.6408$) and **(C)** Collagen 5a1 ($p=0.0191$) stain intensity normalized to Hoechst signal in $MBNL1^{Fl/Fl}$-$Tcf21^{iCre}$ ($n=3$) and $MBNL1^{Fl/Fl}$ ($n=3$) mice 14 days post MI. Dots represent biological replicates, bars represent mean±SEM, unpaired t-tests. **(D)** Heatmap of differentially expressed genes in $MBNL1^{Fl/Fl}$-$Tcf21^{iCre}$ versus $MBNL1^{Fl/Fl}$ cardiac fibroblasts 14 days post MI. Colored bars represent functionally clustered genes: myofibroblast=blue, ECM=red, and bone/cartilage=green. **(E)** Immunofluorescent images and **(F)** quantification of the number of cardiac fibroblasts from $MBNL1^{Fl/Fl}$ and $MBNL1^{Fl/Fl}$-$Tcf21^{iCre}$ mice with αSMA^+ stress fibers as a percentage of the total number counted. αSMA^+ stress fibers (red) and nuclei (blue). Dots represent biological replicates ($n=12$), bars represent mean±SEM, numbers represent $p<0.05$ from ANOVA statistical test with Tukey post hoc comparisons: **1**- Fl Control, **2**- Fl-Cre Control, **3**- Fl TGFβ, **4**- Fl-Cre TGFβ, **5**- Fl AdMBNL, **6**- Fl-Cre AdMBNL1, **7**- Fl AdSRF, **8**- Fl-Cre AdSRF. **(G)** Representative images and **(H)** quantification of contracted collagen gel matrices seeded with tamoxifen-treated $MBNL1^{Fl/Fl}$-$Tcf21^{iCre}$ and $MBNL1^{Fl/Fl}$ cardiac fibroblasts. Yellow rings mark original gel size and white dashed rings show contracted gel size. Dots represent biological replicates ($n=4$), bars represent mean±SEM, numbers represent $p<0.05$ from ANOVA statistical test with Tukey post hoc comparisons: **1**- Fl Control, **2**- Fl-Cre Control, **3**- Fl TGFβ, **4**- Fl-Cre TGFβ, **5**- Fl AdMBNL1, **6**- Fl-Cre AdMBNL1, **7**- Fl AdΔCnA, **8**- Fl-Cre AdΔCnA. (**I-K**) Gene expression (FPKM, fragments per kilobase of transcript) of cell cycle regulators in $MBNL1^{Fl/Fl}$-$Tcf21^{iCre}$ ($n=3$) and $MBNL1^{Fl/Fl}$($n=4$) cardiac fibroblasts 4 days post MI. Dots represent biological replicates, bars represent mean±SEM, unpaired t-test, *$p<0.05$. Related to **Figure 4**.



Supplemental Figure 6: scRNAseq cluster analysis.
(**A-F**) Gene expression of select fibroblast state markers visualized on dimensionality reduction plots. Cell population percentages broken up by genotype in **(G)** Sham and **(H)** 7 day post MI animals, significant differences from NTG represented with *. **(I)** Functional clustering of genes expressed in quiescent fibroblast clusters that significantly expanded in MBNL1$^{Fl/Fl}$-Tcf21^{iCre} mice versus MBNL1$^{Fl/Fl}$ in response MI. The number of genes in each GO category is correlated with the circle size and false discovery rate correlated with color. Gene expression by cluster of **(J)** *Ly6a* and **(K)** *Pdgfra*. Pseudotime analysis of all clusters as visualized by **(L)** UMAP or **(M)** violin plot. Significantly differentially expressed genes between clusters **(N)** MYO-2 versus MYO-1 and **(O)** F-ACT versus MYO-1. Related to **Figure 5**.

Supplemental Figure 7



Supplemental Figure 7: Cardiokines unique to myofibroblast clusters.
(A) Dot plot showing differential expression of classical secretory cardiokines by cluster from scRNAseq. **(B)** Expression of *Fstl1* transcripts immunoprecipitated by MBNL1 in fibroblasts overexpressing MBNL1 versus the IgG control. Dots represent biological replicates, bars represent mean±SEM, unpaired t-test,****p<0.0001**.** Related to **Figure 3 & 5**.

Supplemental Table 1: Primers used for gene expression analysis, Related to STAR methods.

Name	5-3 Seq
GAPDH Human Fwd	GAGTCAACGGATTTGGTCGT
GAPDH Human Rvs	GACAAGCTTCCCGTTCTCAG
18s Human Fwd	GTAACCCGTTGAACCCCATT
18s Human Rvs	CCATCCAATCGGTAGTAGCG
MBNL1 Human Fwd	CTCTGTCCGGTTGACAGGC
MBNL1 Human Rvs	CTGAAAACATTGGCACGGGT
Acta2 Human Fwd	GTGTTGCCCCTGAAGAGCAT
Acta2 Human Rvs	GCTGGGACATTGAAAGTCTCA
18s Mouse Fwd	GTAACCCGTTGAACCCCATT
18s Mouse Rvs	CCATCCAATCGGTAGTAGCG
Acta2 Mouse Fwd	ACCCACCCAGAGTGGAGAAG
Acta2 Mouse Rvs	AGCATCATCACCAGCGAAG
Col1a1 Mouse Fwd	AATGCAATGAAGAACTGGACTG
Col1a1 Mouse Rvs	CCCTCGACTCCTACATCTTCTG
MBNL1 Mouse Fwd	CACTGAAAGGTCGTTGCTCCA
MBNL1 Mouse Rvs	CGCCCATTTATCTCTAACTGTGT
Fstl1 Mouse Fwd	CACGGCGAGGAGGAACCTA
Fstl1 Mouse Rvs	TCTTGCCATTACTGCCACACA
Sox9 Mouse Fwd	TATCTTCAAGGCGCTGCAA
Sox9 Mouse Rvs	TCGGTTTTGGGAGTGGTG
Cdkn1a Mouse Fwd	GCAGACCAGCCTGACAGATTT
Cdkn1a Mouse Rvs	CTGACCCACAGCAGAAGAGG