Supplemental Figure and Movie Legends

Supplemental Fig. 1. Conditional targeting of the *Myocd* gene. (A) Schematic representation of the myocardin protein and the conditional gene targeting strategy utilized in this manuscript. Schematic representation of myocardin protein is shown in the upper panel. The locations of the RPEL, Basic, Glutamine-rich (Q), SAP and transcriptional activation (TD) domains and leucine zipper (LZ) are shown. A schematic representation of exons 7-10 (rectangles) of the *Myocd* gene including the location restriction enzyme sites utilized to genotype ES cells and mice. The position of the DNA probe used for Southern blot analysis is indicated below (black rectangle). The targeted allele contains the PGK-neomycin resistance (neo) cassette and loxP sites (triangles) flanking exon 8. **(B)** PCR genotype analysis showing the expected bands corresponding to the wild-type allele (+) and *Myocd* null allele (-) following $Myocd^{+/-}$ germline transmission. **(C)** Southern blot analysis of mouse genomic DNA demonstrating the 11.4-kb wild-type allele (+) and the 5.4-kb null allele. **(D)** RT-PCR was performed with cardiac mRNA to identify products corresponding to WT (+/+) and null (-/-) alleles.

Supplemental Fig. 2. Nkx2.5 and Mef2c expression in the hearts of E8.5-9.5 $Myocd^{-/-}$ null embryos and *E9.5-12.5* $Nkx2\text{-}5Cre^{+}/Myocd^{F/F}$ conditional mutant embryos. (A-H) Immunocytochemical analyses of transverse histological sections prepared from E8.5 wild-type (WT) control and $Myocd^{-/-}$ mutant embryos was performed with anti-Nkx2.5 and anti-Mef2c antibodies. Abundant nuclear expression (green signal) of Nkx2.5 and Mef2c is observed throughout the primitive heart tube of WT and $Myocd^{-/-}$ embryos at E8.5, but expression of both factors is extinguished in E9.5 $Myocd^{-/-}$ embryos. **(I-X)** Immunocytochemical analyses of transverse histological sections prepared from E9.5-12.5 $Myocd^{F/F}$ (Control) and $Nkx2.5Cre^{+}/Myocd^{F/F}$ conditional mutant embryos was performed with anti-Nkx2.5 and anti-

Mef2c antibodies and sections were counterstained with DAPI (blue nuclear stain). Abundant nuclear expression Nkx2.5 (green signal) and Mef2c (red or green signal) is observed in control and and conditional mutant hearts at E9.5, but expression of both factors is extinguished in the hearts of E9.5-12.5 *Nkx2.5Cre*$^{+}$*/Myocd*$^{F/F}$ conditional mutant embryos.

Supplemental Movie 1. 3 dimensional (3D) high resolution map of the heart of E9.5 control embryo. E9.5 embryos were harvested and genotyped from intercrosses of *Myocd*$^{+/-}$ mice. Whole-mount immunostaining was performed with monoclonal anti-MLC2v primary antibody and HRP-conjugated IgG secondary antibody to identify cardiomyocytes populating the embryonic heart. High-resolution optical mapping studies were performed on an Olympus BX51WI microscope equipped with a high-speed CMOS camera. This movie file shows the 3 dimensional (3D) reconstructed image of a wild-type embryo demonstrates that the primitive heart tube (orange stain) has completed looping morphogenesis.

Supplemental Movie 2. 3D high resolution map of the heart of E9.5 *Myocd*$^{-/-}$ mutant embryo. 3 dimensional (3D) high resolution optical mapping was performed on E9.5 embryos immunostained with anti-MLC2v antibody as described under Experimental Procedures. This movie file shows the 3D reconstructed image of a *Myocd*$^{-/-}$ mutant embryo demonstrating that the primitive heart tube (orange stain) has completed looping morphogenesis.

Supplemental Movie 3. Echocardiogram showing the heart of E9.5 control embryo (2-chamber view). 2-dimensional (2D)-echocardiogram showing the common atria and ventricle (corresponding to 4 chamber view of later stages of development) taken of E9.5 *Myocd*$^{+/F}$ control mouse heart demonstrating normal ventricular dimensions and function. Scale in mm is shown above and to the right of the echocardiogram. ECG recording is shown below the

echocardiogram. The frame rate has been slowed to 30 frames/sec to facilitate visualization of cardiac contraction and relaxation.

Supplemental Movie 4. Echocardiogram showing the heart of E9.5 control embryo (short axis view). 2D echocardiogram short-axis view taken of E9.5 *Myocd*$^{+/F}$ control mouse heart demonstrating normal left ventricular dimensions and function.

Supplemental Movie 5. Echocardiogram showing the heart of E9.5 *Myocd*$^{-/-}$ mutant embryo (2-chamber view). 2D echocardiogram showing the common atria and ventricle (corresponding to 4 chamber view at later stages of development) taken of a E9.5 *Myocd*$^{-/-}$ mutant mouse heart demonstrating bradycardia and severely depressed ventricular function. A small pericardial effusion is also present. These changes are indicative of heart failure at this stage of embryonic development.

Supplemental Movie 6. Echocardiogram showing the heart of E9.5 *Myocd*$^{-/-}$ mutant embryo (short axis view). 2D echocardiogram short-axis view taken of a E9.5 *Myocd*$^{-/-}$ mutant mouse heart demonstrating bradycardia and severely depressed ventricular function. These changes are indicative of heart failure at this stage of embryonic development.

Supplemental Movie 7. Phase-contrast videomicroscopy showing the heart of wild-type control embryo. Hearts isolated from E9.5 control (n = 10) and *Myocd*$^{-/-}$ mutant (n = 10) embryos were cultured ex vivo in control conditioned media (n = 5) or Bmp10-conditioned

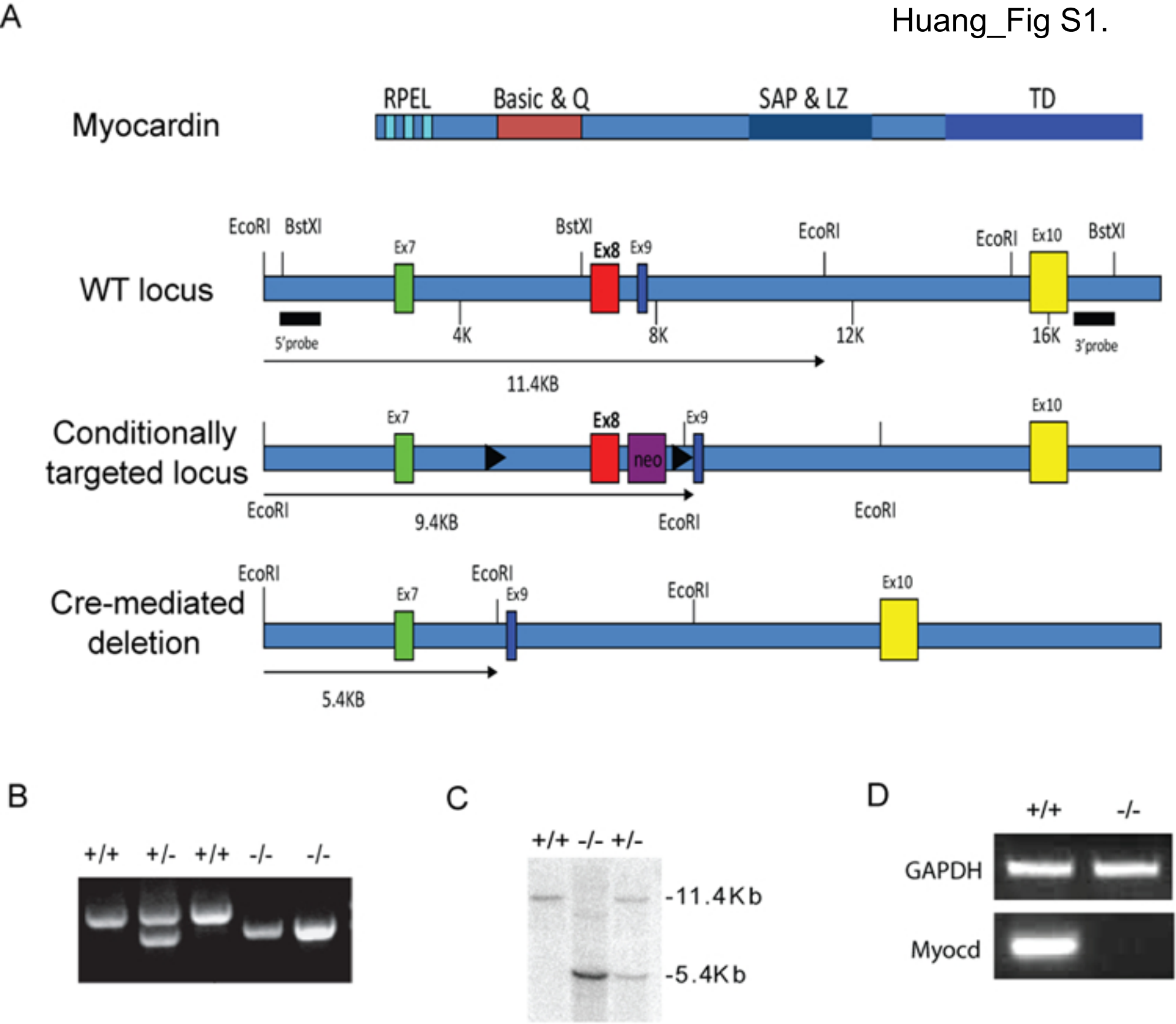
media (n = 5) as described in *Methods*. This video clip shows a control heart cultured in control conditioned media 24-h post-isolation beating vigorously in culture.

Supplemental Movie 8. Phase-contrast videomicroscopy showing the heart of *Myocd*$^{-/-}$ mutant embryo grown in control conditioned media. Hearts isolated from E9.5 control (n = 10) and *Myocd*$^{-/-}$ mutant (n = 10) embryos were cultured ex vivo in control conditioned media (n = 5) or Bmp10-conditioned media (n = 5) as described in *Methods*. This video clip shows a *Myocd*$^{-/-}$ heart cultured in control conditioned media 24-h post-isolation. The heart was much smaller than hearts isolated from control embryos and beating was much less vigorous and much slower.

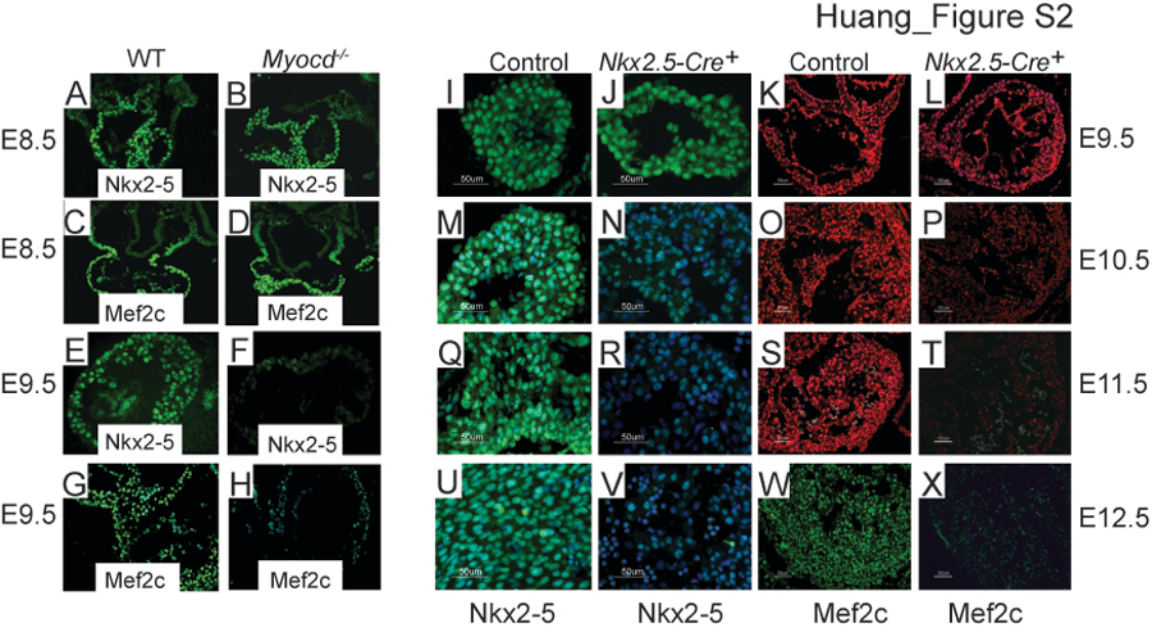
Supplemental Movie 9. Phase-contrast videomicroscopy showing the heart of *Myocd*$^{-/-}$ mutant embryo grown in Bmp10-conditioned media. Hearts isolated from E9.5 control (n = 10) and *Myocd*$^{-/-}$ mutant (n = 10) embryos were cultured ex vivo in control conditioned media (n = 5) or Bmp10-conditioned media (n = 5) as described in *Methods*. This video clip shows a *Myocd*$^{-/-}$ heart cultured in Bmp10-conditioned media 24-h post-isolation. The heart was much smaller than hearts isolated from control embryos, but beating more rapid and vigorous compared to *Myocd*$^{-/-}$ mutant hearts cultured in control conditioned media.

Supplemental Movie 10. Phase-contrast videomicroscopy showing the heart of *Myocd*$^{-/-}$ mutant embryo grown in Bmp10-conditioned media. Hearts isolated from E9.5 control (n = 10) and *Myocd*$^{-/-}$ mutant (n = 10) embryos were cultured ex vivo in control conditioned media (n = 5) or Bmp10-conditioned media (n = 5) as described in *Methods*. This video clip shows a

second *Myocd*$^{-/-}$ heart cultured in Bmp10-conditioned media 24-h post-isolation. The heart was much smaller than hearts isolated from control embryos, but beating more rapid and vigorous compared to *Myocd*$^{-/-}$ mutant hearts cultured in control conditioned media.


A
Myocardin
RPEL
Basic & Q
SAP & LZ
TD
WT locus
EcoRI
BstXI
Ex7
BstXI
Ex8
Ex9
EcoRI
EcoRI
Ex10
BstXI
5'probe
4K
8K
12K
16K
3'probe
11.4KB
Conditionally targeted locus
Ex7
Ex8
Ex9
Ex10
neo
EcoRI
9.4KB
EcoRI
EcoRI
Cre-mediated deletion
EcoRI
EcoRI
Ex7
Ex9
EcoRI
Ex10
5.4KB
B
+/+ +/- +/+ -/- -/-
C
+/+ -/- +/-
-11.4Kb
-5.4Kb
D
+/+ -/-
GAPDH
Myocd

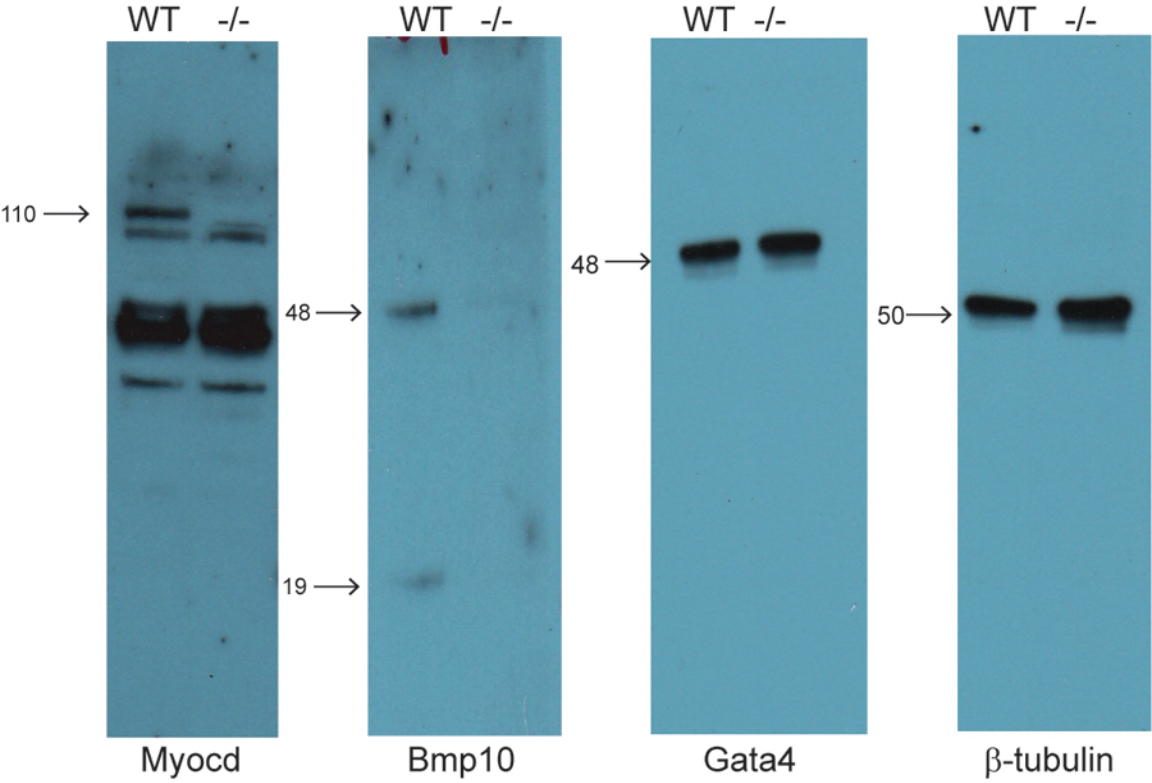
Huang_Figure S2



Supplemental Table 1. List of primary antibodies utilized for immunohistochemical analyses of myocardin null and conditional mutant embryos.

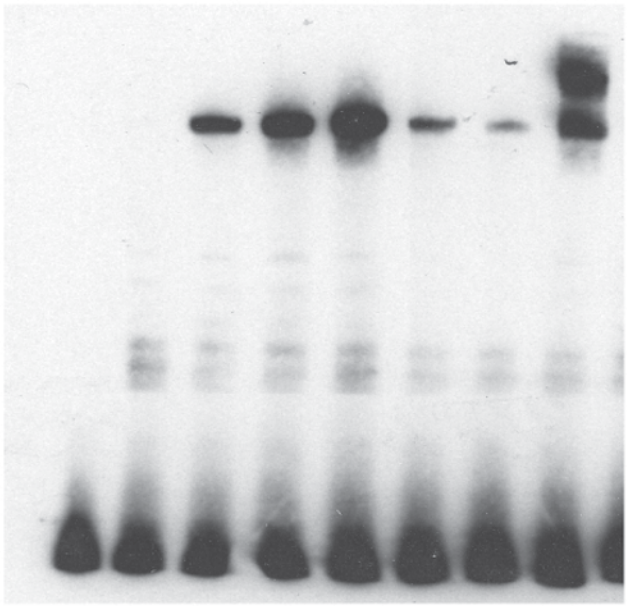
Antibody Name	**Catalog #**	**Company**
BMP10	MAB6038	R&D Systems
Cre	clone7-23	Sigma-Aldrich
SMA	1A4	Sigma-Aldrich
SM22α	Ab10135	Abcam
p57Kip2	KP39	Lab Vision
Phospho-Histone H3 **(**Ser10)	9701	Cell Signaling Technology
SRF	G-20	Santa Cruz
alpha actinin	EA-53	Sigma
cardiac troponin T	ab8295	Abcam
PCNA	PC10	Biocare
cardiac-α-actin	Ac1-20.4.2	American Research Products
troponin C	E7	Santa Cruz
Nkx2.5	N19	Santa Cruz
GATA4	c-20	Santa Cruz
p21	F-5	Santa Cruz
cyclin D2	M-20	Santa Cruz
BrdU	G3G4 (AntiBrdUrd)	Hybridoma Bank
MLC2v	ALX-BC-1150	AXXORA
tropomyosin	TM311	Sigma
MEF-2C	C-21	Santa Cruz
MF20		Hybridoma Bank
β-tubulin	ab6046	Abcam

Huang_Original Gels Fig. 6E



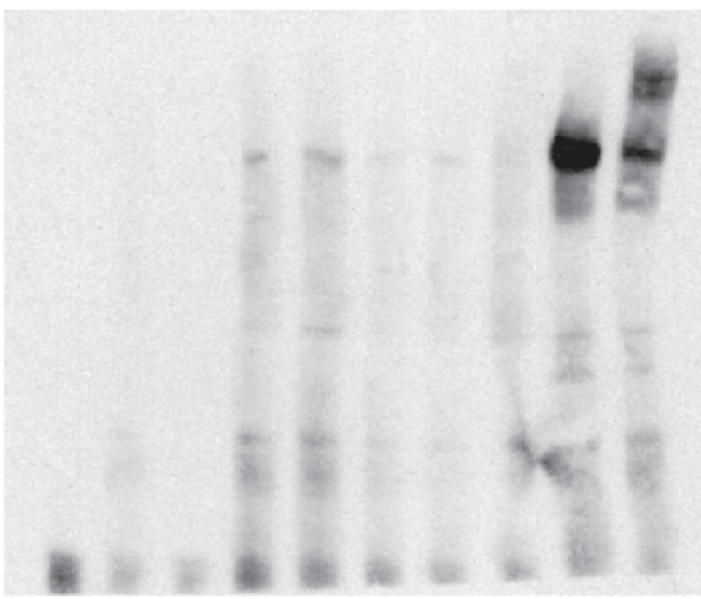
Huang_Original EMSA Fig. 7B

Myocardin	-	-	0.1	0.5	1.0	1.0	1.0	1.0
SRF	-	-	-	-	-	-	-	-
SRF Ab	-	-	-	-	-	-	-	+
Comp (ng)	-	-	-	-	-	10	50	+



1 2 3 4 5 6 7 8

SRF	-	-	0.1	0.5	1.0	1.0	1.0	-	1.0	1.0
SRF antisera	-	-	-	-	-	-	-	-	-	+
Competitor	-	-	-	-	-	10	50	-	-	-



9 10