Cell Reports, Volume *32*

Supplemental Information

Tp53 Suppression Promotes Cardiomyocyte Proliferation during Zebrafish Heart Regeneration

Adam Shoffner, Valentina Cigliola, Nutishia Lee, Jianhong Ou, and Kenneth D. Poss

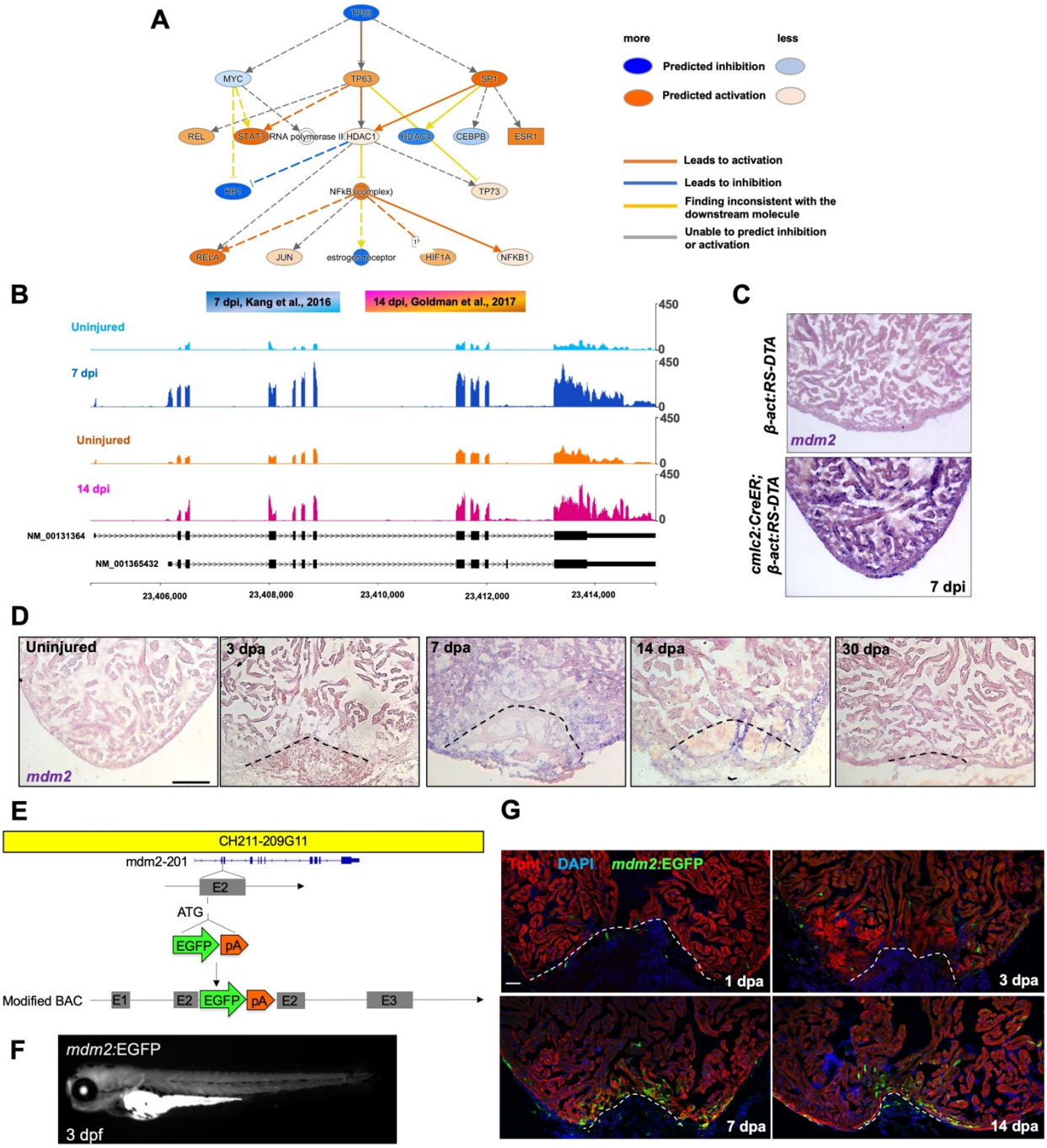
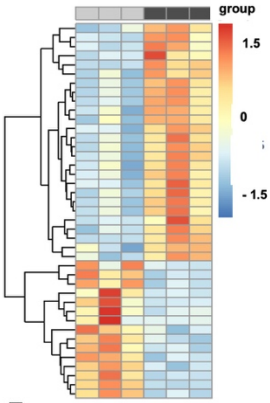
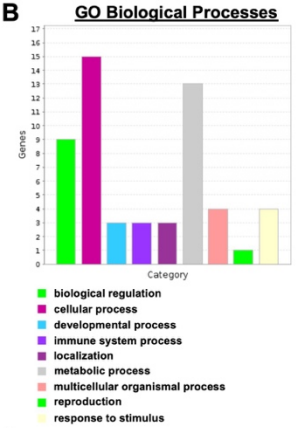

A
TP63
MYC
TP63
SP1
REL
STAT3
RNA polymerase II
HDAC1
HDAC2
CEBPB
ESR1
RB1
NFkB (complex)
TP73
RELA
JUN
estrogen receptor
HIF1A
NFKB1
more
less
Predicted inhibition
Predicted activation
Leads to activation
Leads to inhibition
Finding inconsistent with the downstream molecule
Unable to predict inhibition or activation
B
7 dpi, Kang et al., 2016
14 dpi, Goldman et al., 2017
Uninjured
7 dpi
Uninjured
14 dpi
450
0
NM_00131364
NM_001365432
23,406,000
23,408,000
23,410,000
23,412,000
23,414,000
C
β-act:RS-DTA
mdm2
cmlc2:CreER;
β-act:RS-DTA
7 dpi
D
Uninjured
3 dpa
7 dpa
14 dpa
30 dpa
mdm2
E
CH211-209G11
mdm2-201
E2
ATG
EGFP
pA
Modified BAC
E1
E2
EGFP
pA
E2
E3
F
mdm2:EGFP
3 dpf
G
ToM
DAPI
mdm2:EGFP
1 dpa
3 dpa
7 dpa
14 dpa

Figure S1 (related to Figure 1). *mdm2* expression is induced after heart injury. (A) Analysis of transcriptome data of 7 dpi regenerating zebrafish published by Kang et al., 2016. Solid arrows indicate direct and dashed arrow indicates indirect interactions. Predictions on the direction and intensity of activation and inhibition by TP53 were made by IPA knowledgebase, based on published literature. (**B**) Genome browser tracks indicating expression of *mdm2* 7 days and 14 days after induced CM ablation (dpi), versus uninjured ventricles. **(C)** In situ hybridization for *mdm2* (violet) in sections of control (no CreER) and injured (7 dpi) ventricles, 7 days after tamoxifen adminstration. **(D)** In situ hybridization for *mdm2* in ventricular sections at different time points after resection of the ventricular apex (dpa). Dashed lines approximate the resection plane. Scale bars: 100 μm. **(E)** Cartoon showing the *mdm2:EGFP* BAC transgene. **(F)** Larval *mdm2*-directed fluorescence, shown at 3 day post fertilization (dpf). **(G)** Images showing *mdm2*-directed EGFP at different time points after resection of the ventricular apex. Tnnt marks CMs. Dashed lines approximate resection plane. Scale bars: 100 μm.

A Uninj *tp53*$^{-/-}$ vs *tp53*$^{+/+}$



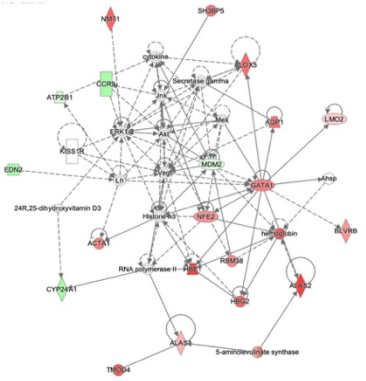
B GO Biological Processes



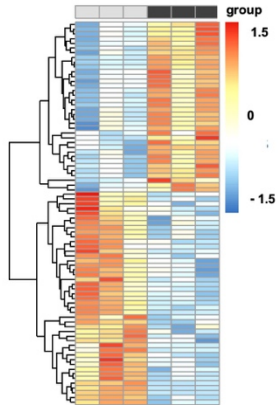
C Selected Canonical Pathways

Ingenuity Canonical Pathways	p-value
Tetrapyrrole Biosynthesis II	0,04
Heme Biosynthesis II	0,05
Iron homeostasis signaling pathway	0,12
Heme Degradation	0,21
Calcium Transport I	0,27
Leukotriene Biosynthesis	0,29
HOTAIR Regulatory Pathway	0,30
Clathrin-mediated Endocytosis Signaling	0,34
Calcium Signaling	0,35
Apelin Liver Signaling Pathway	0,36
Notch Signaling	0,40
VDR/RXR Activation	0,50
VEGF Signaling	0,01
HIF1α Signaling	0,53
RhoA Signaling	0,56
PI3K/AKT Signaling	0,62

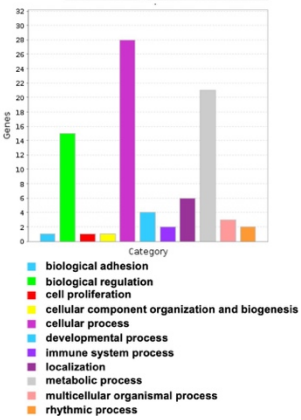
D Top Gene Network



E 7 dpi *tp53*$^{-/-}$ vs *tp53*$^{+/+}$



F GO Biological Processes



G Selected Canonical Pathways

Ingenuity Canonical Pathways	p-value
Sirtuin Signaling Pathway	0,11
Oxidative Ethanol Degradation III	0,12
Ethanol Degradation IV	0,14
Glutamine Biosynthesis I	0,16
D-mannose Degradation	0,16
Ethanol Degradation II	0,17
4-hydroxyproline Degradation I	0,20
Stearate Biosynthesis I (Animals)	0,21
Proline Degradation	0,23
Arginine Degradation I (Arginase Pathway)	0,25
Acetate Conversion to Acetyl-CoA	0,25
Glycine Cleavage Complex	0,28
Leucine Degradation I	0,28
Fatty Acid Activation	0,31
VDR/RXR Activation	0,59
HIPPO signaling	0,60

H Top Gene Network

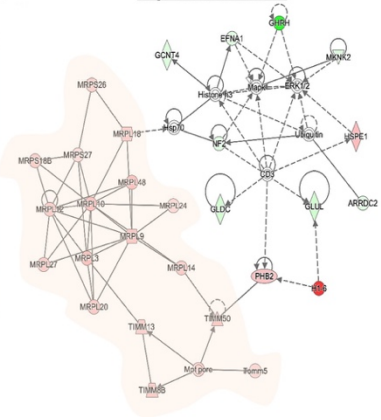


Figure S2 (related to Figure 2). *tp53*$^{+/+}$ and *tp53*$^{-/-}$ transcriptomic data. (A) Heatmap showing 42 genes differentially modulated in uninjured ventricles (Table S5). Black bars, *tp53*$^{-/-}$. Grey bars, *tp53*$^{+/+}$. **(B, C)** Barplot showing different biological processes assessed by GO analysis **(B)**, and a list of selected differentially modulated canonical pathways assessed by Ingenuity Pathway Analysis **(C;** Table S7**)**. **(D)** Top gene network composed by genes differentially modulated between uninjured *tp53*$^{-/-}$ and *tp53*$^{+/+}$ ventricles. **(E)** Heatmap showing 82 genes differentially modulated between *tp53*$^{-/-}$ and *tp53*$^{+/+}$ ventricles at 7 days after genetic CM ablation **(**Table S6**)**. **(F, G)** Barplot showing different biological processes assessed by GO analysis **(F)**, and a list of selected differentially modulated canonical pathways assessed by Ingenuity Pathway Analysis **(G;** Table S8**)**. **(H)** Top gene network composed by genes differentially modulated in regenerating *tp53*$^{-/-}$ and *tp53*$^{+/+}$ ventricles.

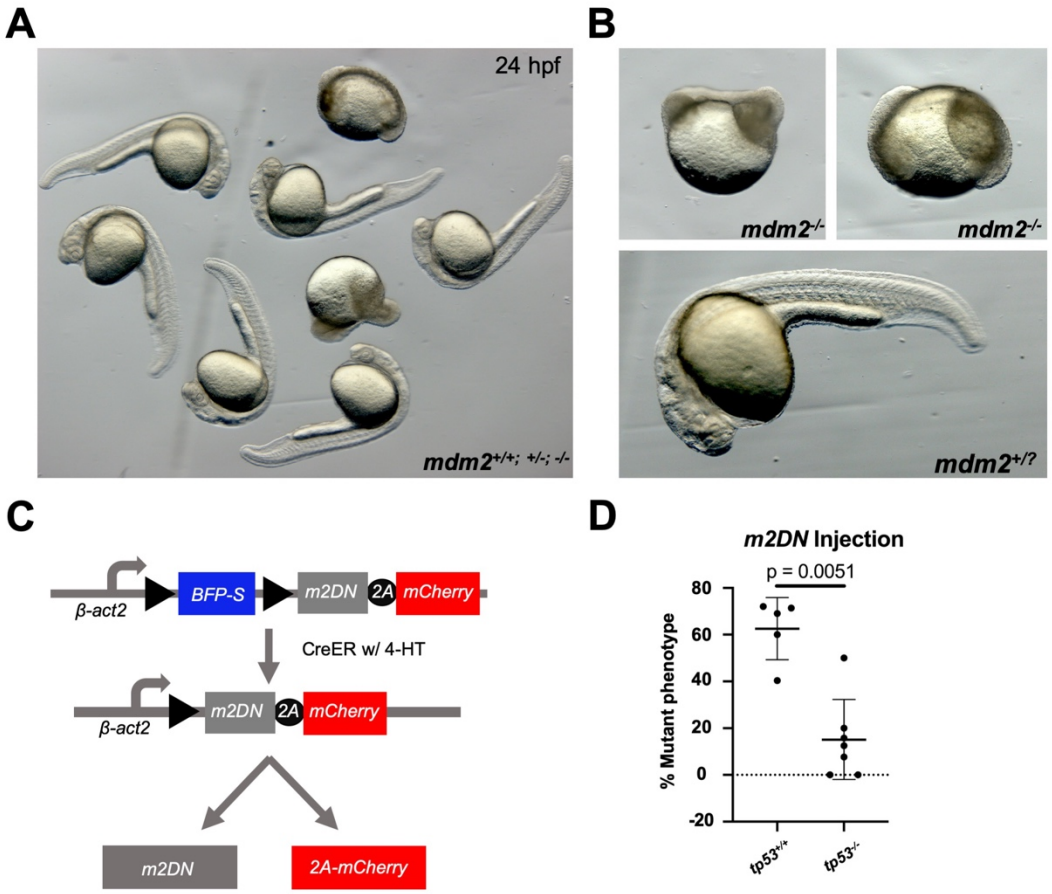

A
24 hpf
mdm2+/+; +/-; -/-
B
mdm2-/-
mdm2-/-
mdm2+/?
C
β-act2
BFP-S
m2DN
2A
mCherry
CreER w/ 4-HT
β-act2
m2DN
2A
mCherry
m2DN
2A-mCherry
D
m2DN Injection
p = 0.0051
% Mutant phenotype
80
60
40
20
0
-20
tp53+/+
tp53-/-
E
24 hpf
tp53-/-
F
24 hpf
tp53+/+

Figure S3 (related to Figure 2). Effects of *mdm2* deletion mutations and *dn-mdm2* construct expression in zebrafish embryos. (A, B) Images showing *mdm2*$^{+/+}$, *mdm2*$^{+/-}$, and *mdm2*$^{-/-}$ embryos from an *mdm2*$^{+/-}$ cross at 24 hours post fertilization (hpf). **(C)** Cartoon schematic of *β-act2:BSm2DN* transgene. Mutations within or loss of this Mdm2 critical domain have been demonstrated both to phenocopy and fail to rescue *mdm2* mutant mice or zebrafish. **(D)** Percentage of larvae recapitulating the *mdm2*$^{-/-}$ mutant phenotype upon injection of *m2DN* mRNA into single-cell embryos. This construct had limited effects in *tp53* mutant embryos. **(E, F)** Images showing *m2DN*-injected *tp53*$^{-/-}$ and *tp53*$^{+/+}$ larvae 24 hours post fertilization (24 hpf). Asterisks indicate embryos with gross morphological defects.

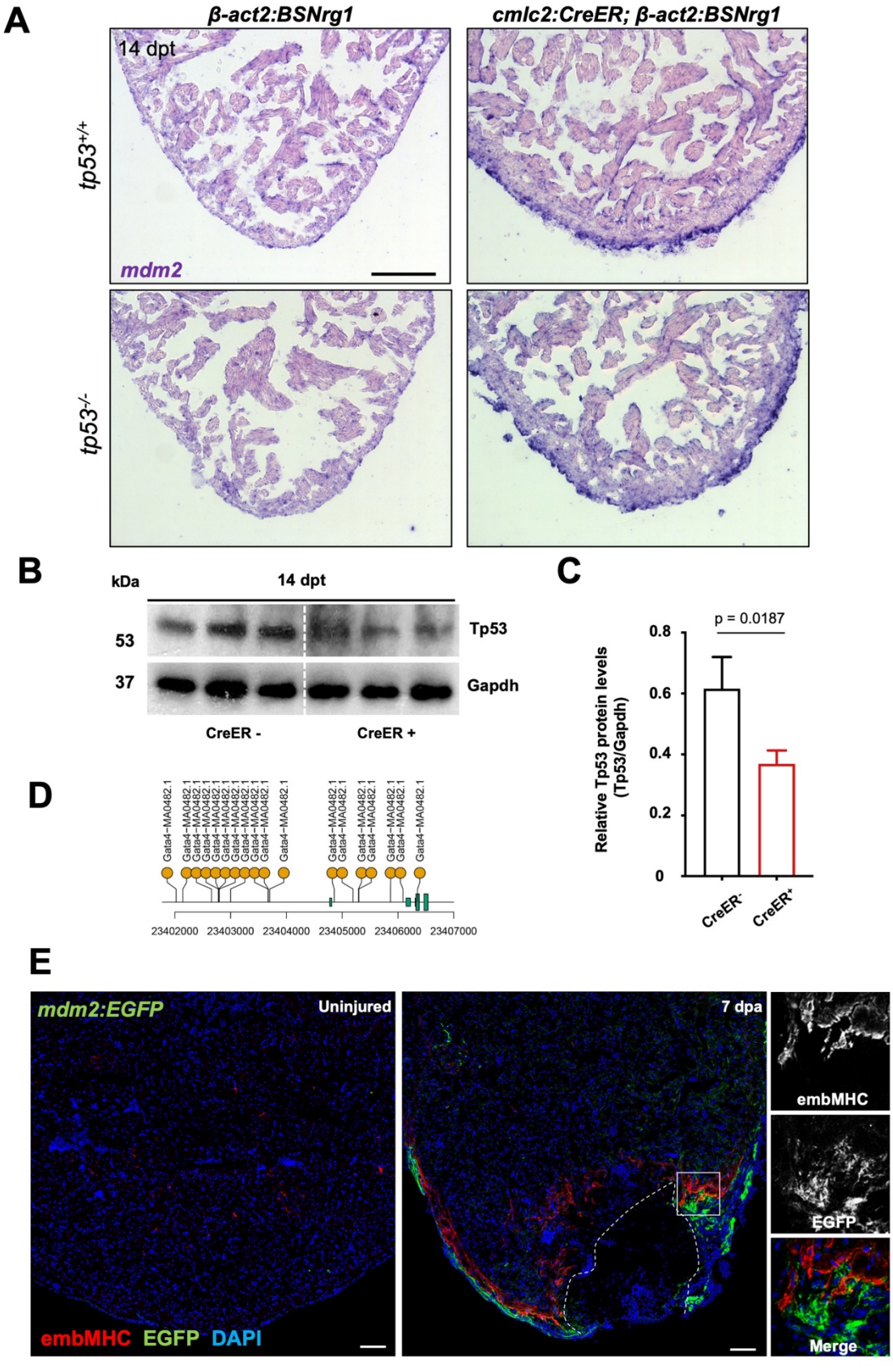

A
β-act2:BSNrg1
cmlc2:CreER; β-act2:BSNrg1
14 dpt
mdm2
tp53+/+
tp53-/-
B
kDa
14 dpt
53
37
Tp53
Gapdh
CreER -
CreER +
C
p = 0.0187
Relative Tp53 protein levels (Tp53/Gapdh)
0.8
0.6
0.4
0.2
0
CreER-
CreER+
D
Gata4-MA0482.1
23402000
23403000
23404000
23405000
23406000
23407000
E
mdm2:EGFP
Uninjured
7 dpa
embMHC
EGFP
DAPI
Merge

Figure S4 (related to Figures 3 and 4). Modulation of Tp53 and *mdm2* in Nrg1-overexpressing hearts, and localization of embMHC with *mdm2*-directed fluorescence. (A) In situ hybridization for *mdm2* on ventricular sections with (right) or without (left) Nrg1 overexpression in $tp53^{+/+}$ and $tp53^{-/-}$ animals. *mdm2* is similarly induced in both *tp53* genotypes, at the peripheral edge of cortical muscle. Scale bars: 100 μm. **(B)** Western blot to detect Tp53, from control cardiac proteins and proteins extracted 14 days after Nrg1 induction. **(C)** Quantification of western blot protein bands. Eight to 10 hearts were pooled per sample. Data are represented as mean ± SEM (unpaired t-test). **(D)** Schematic of the *mdm2* promoter region showing potential binding sites for the transcription factor Gata4. Region shown is from 3 kb upstream to 2,226 bp downstream of the *mdm2* transcriptional start site. **(E)** Immunofluorescence staining for EGFP and embMHC at 7 dpa indicates co-expression but not co-localization of the two markers. Dashed line indicates approximate injury plane. Right panels are high-mag views of box. Scale bars: 50 μm.