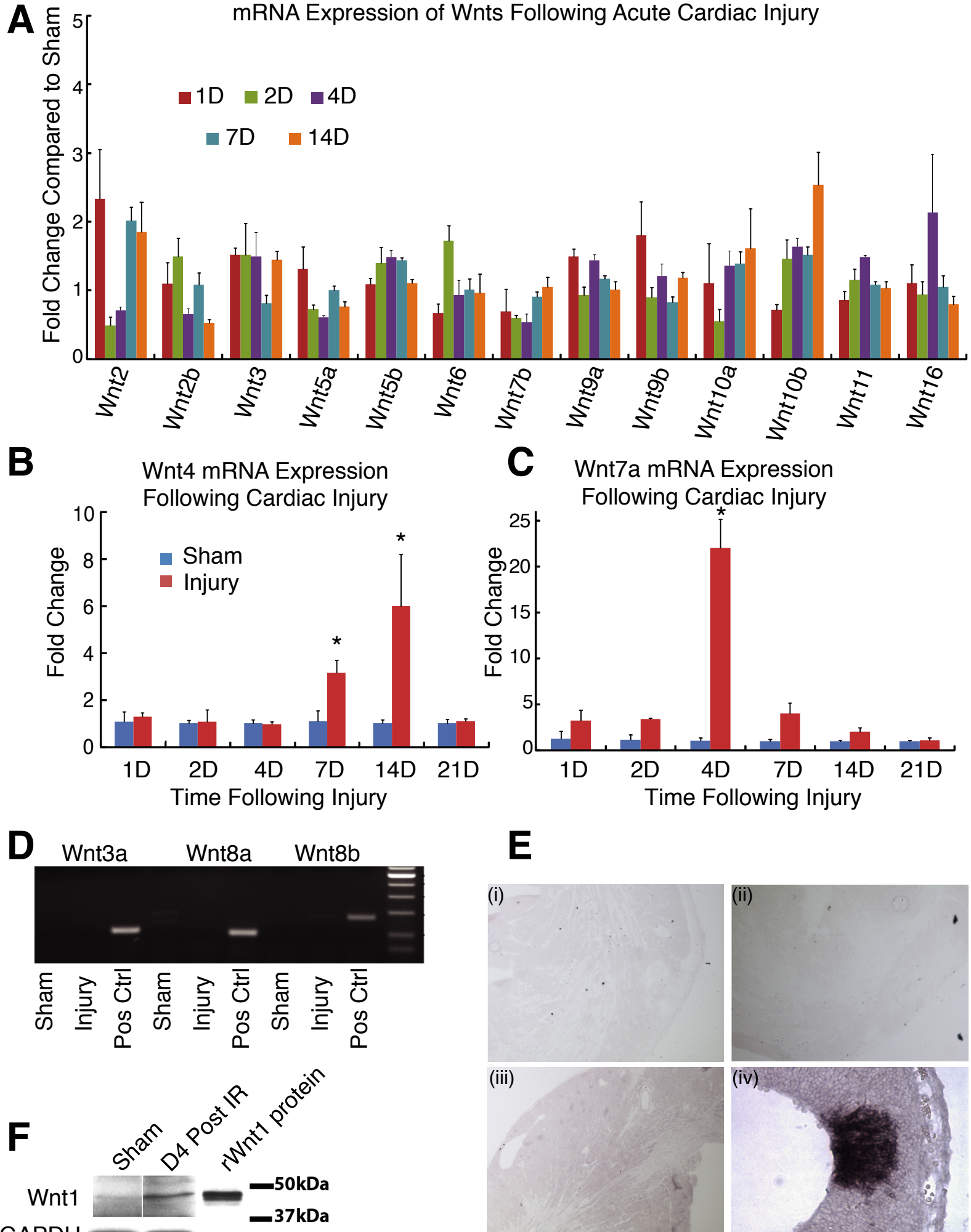

A
mRNA Expression of Wnts Following Acute Cardiac Injury
Fold Change Compared to Sham
1D
2D
4D
7D
14D
Wnt2
Wnt2b
Wnt3
Wnt5a
Wnt5b
Wnt6
Wnt7b
Wnt9a
Wnt9b
Wnt10a
Wnt10b
Wnt11
Wnt16
B
Wnt4 mRNA Expression
Following Cardiac Injury
Fold Change
Sham
Injury
Time Following Injury
C
Wnt7a mRNA Expression
Following Cardiac Injury
Fold Change
Time Following Injury
D
Wnt3a
Wnt8a
Wnt8b
Sham
Injury
Pos Ctrl
E
(i)
(ii)
(iii)
(iv)
F
Sham
D4 Post IR
rWnt1 protein
Wnt1
GAPDH
50kDa
37kDa

FIGURE S1. Expression of Wnts after acute cardiac injury

(**A**) Screening of Wnts at different time points following acute cardiac injury (**B**) Expression of Wnt4 and (**C**) Wnt7A shows temporally separated peaks (**D**) Wnts that are not expressed in the heart with/without injury; PosCtrl refers to ES cells that express these Wnts (**E**) probe controls for ISH, (i-ii) D2 and D10 Sense controls for Wnt1 ISH (iii) scrambled control (iv) positive control of section of mouse embryonic brain (n=8 animals/group,*$p<0.05$ compared to sham; mean±SEM) (**F**) Western Blot for Wnt1 on dissected injured region of heart isolated 4 days after ischemia-reperfusion injury. Recombinant Wnt1 protein is run on the last lane for a positive control. Scale bar: 100μm

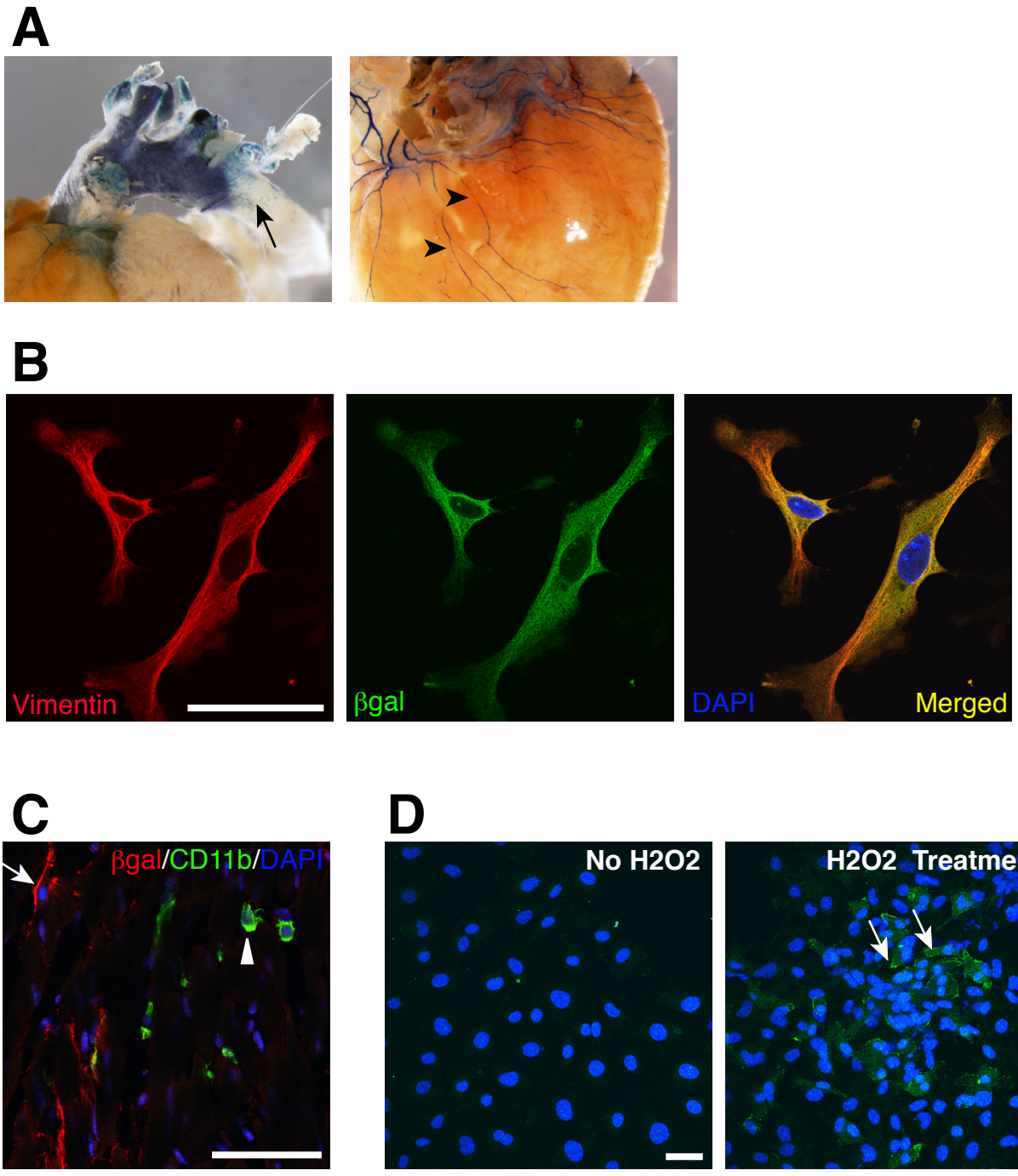


FIGURE S2. Expression of lacZ in hearts of Wnt1Cre/R26R^{lacZ} mice
(A) X gal staining of heart in whole mount demonstrates staining in the aortic arch (arrow) and cardiac nerves (arrowheads) **(B)** Phenotype of βgal positive cells (isolated 2.5 days after cardiac injury) stained with antibodies against βgal (green) and vimentin (red). Nuclei stained with DAPI (blue) with corresponding overlay image showing cells co-localizing βgal and fibroblast marker.**(C)** Section of heart harvested 2 days after injury stained with antibodies against βgal (red) and CD11b (green) **(D)**Wnt1 staining of epicardial cells 16h after H2O2 treatment. Scale bar: 50μm.

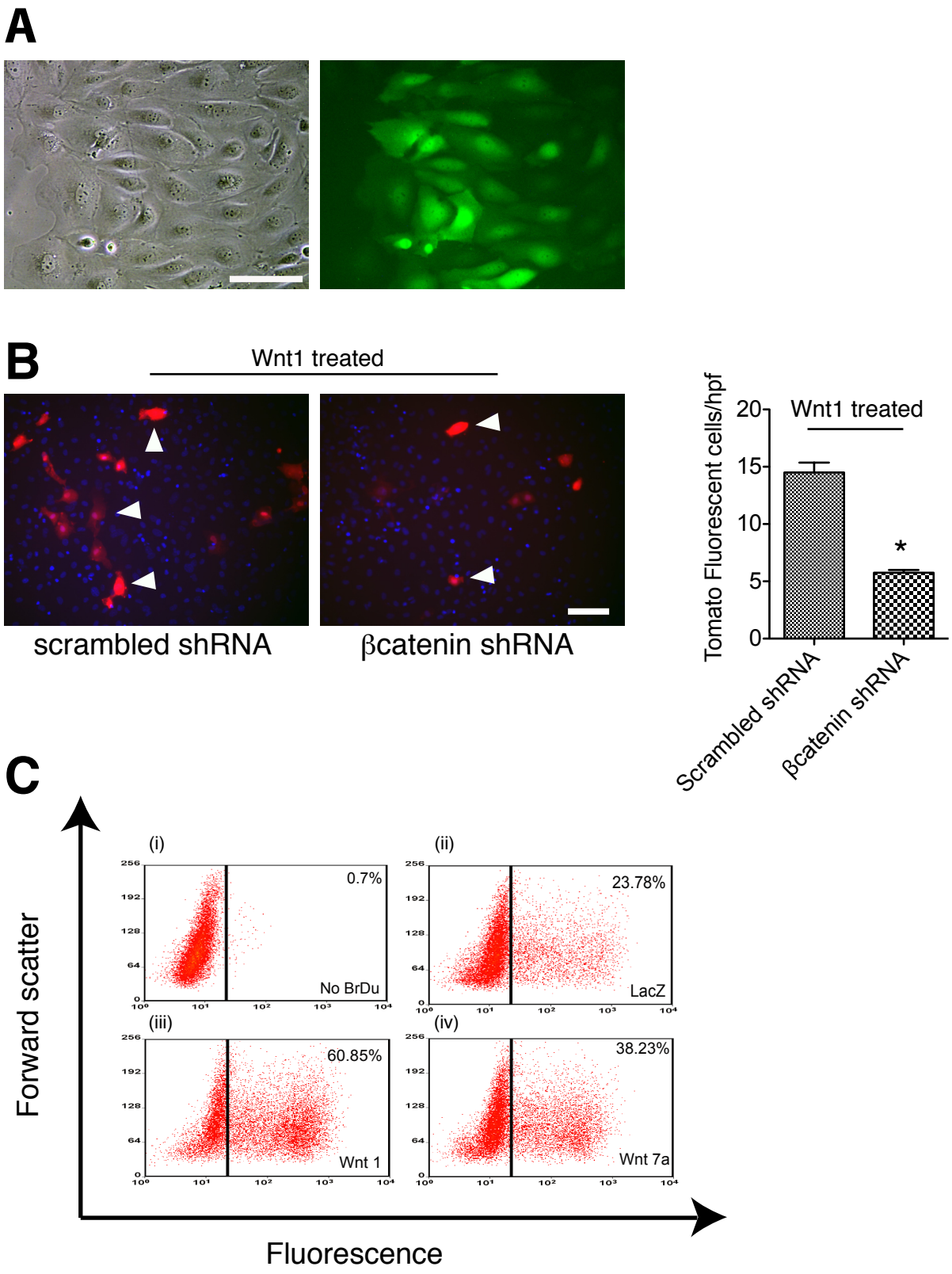
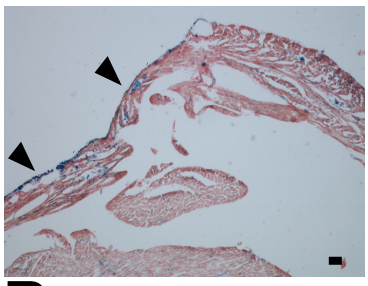
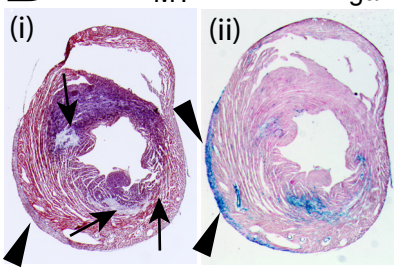
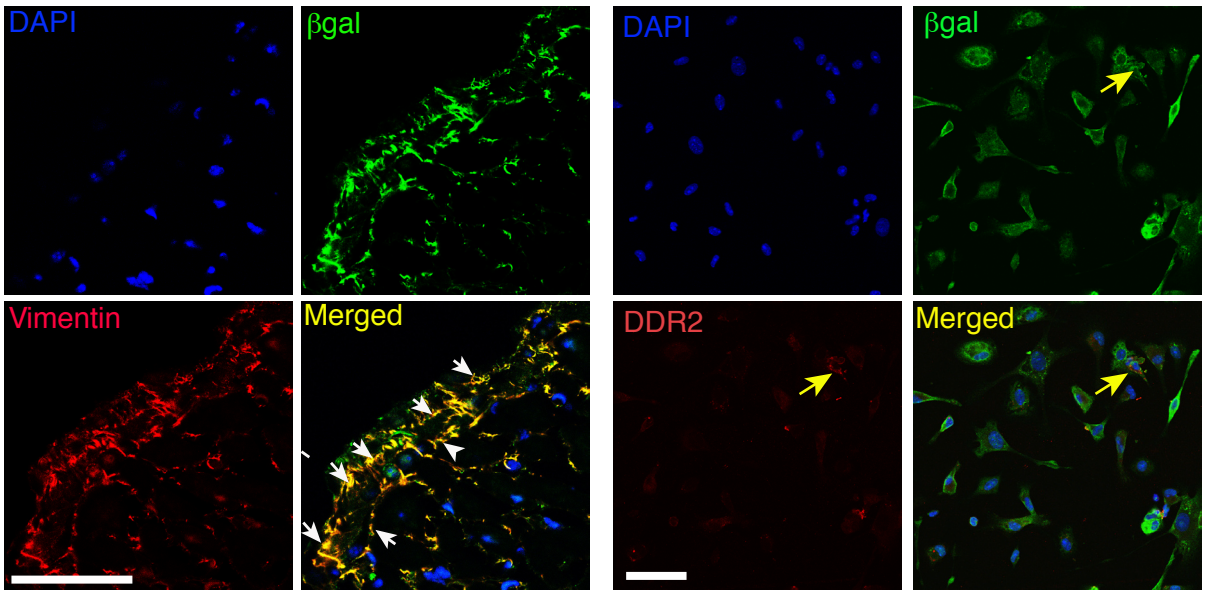
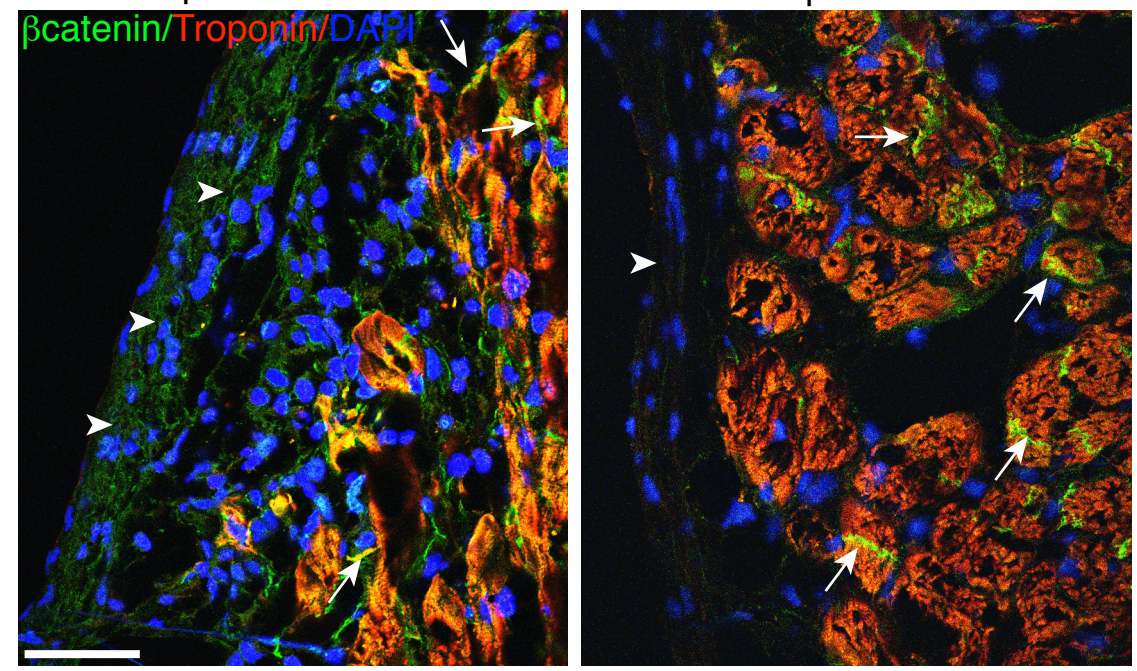


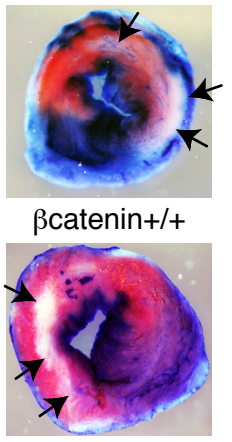
FIGURE S3. Wnt1/βcatenin effects on EMT and cardiac fibroblast proliferation in-vitro
(A, left panel) Epicardial cells isolated from embryonic heart following **(A, right panel)** transduction with lentiviral shRNA scrambled vector co-expressing GFP **(B)** Epicardial cells from Col1a2CreER(T)/R26R^{tdtomato} infected with (left panel) scrambled lentiviral shRNA or (right panel) βcatenin lentiviral shRNA. Both groups of cells were treated with Wnt1 and tamoxifen. Quantitation of tomato fluorescent cells/high power field shown on right (*P<0.05, mean±S.E.M, n=3). **(C)** Wnt1 effects on cardiac fibroblast proliferation determined by BrdU uptake. Flow cytometry showing (i) No BrdU control, and following over-expression of (ii) lacZ, (iii) Wnt1 or (iv) Wnt7a. Scale bar: 100μm




B
MT
Xgal
(i)
(ii)


C
DAPI
βgal
Vimentin
Merged
D
DAPI
βgal
DDR2
Merged


E
βcatenin +/+
Wt-1 βcatenin KO
βcatenin/Troponin/DAPI


G
βcatenin+/+
Wt-1βcatenin KO
IA/AAR (%)
40
30
20
10
0
n.s
βcatenin+/+
Wt-1 βcatenin KO

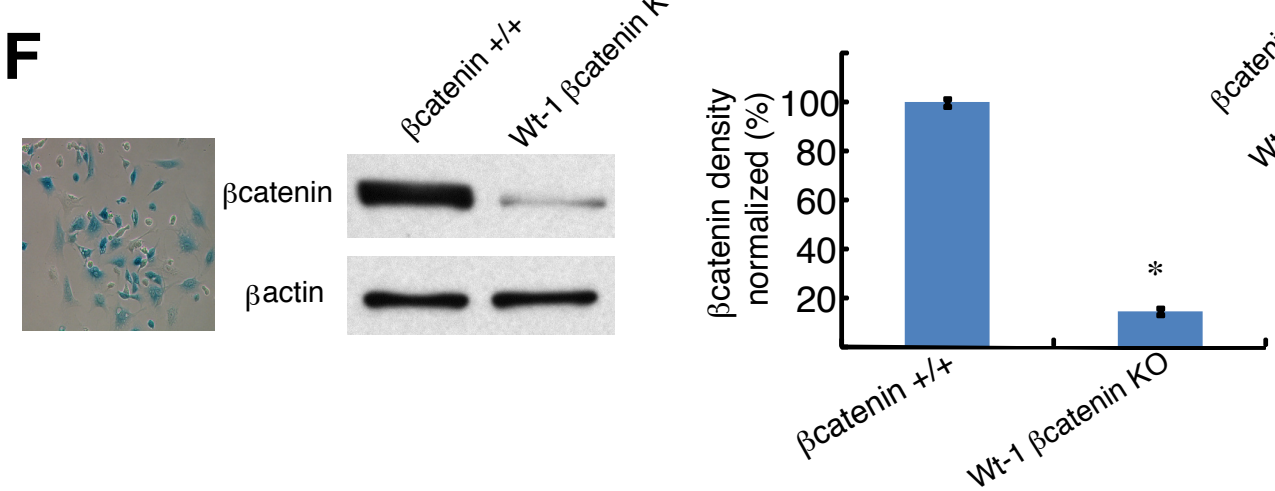

F
βcatenin +/+
Wt-1 βcatenin KO
βcatenin
βactin
βcatenin density normalized (%)
100
80
60
40
20
*
βcatenin +/+
Wt-1 βcatenin KO

FIGURE S4. The epicardium in Wt-1Cre/R26R^{lacZ} mice

(**A**) X gal staining of Wt-1Cre/R26R^{lacZ} mice shows lacZ expressing epicardial cells over the right ventricle (**B**) (i)Masson trichrome and (ii)Xgal staining of heart section 5 days following injury (arrows show area of injury and arrowheads show epicardial expansion (**C**) Staining of expanded epicardium of Wt-1Cre/R26R^{lacZ} mice 5 days post injury with βgalactosidase and vimentin antibodies shows cells that express both antigens (arrows) (**D**) Immunostaining for βgalactosidase and DDR2 in cells isolated from Wt-1Cre/ βcatenin$^{fl/fl}$/R26R^{lacZ} mouse heart 5 days post injury demonstrates rare Cre positive cells expressing DDR2 (yellow arrow) (**E**) Staining of heart section of Wt-1Cre/βcatenin$^{fl/fl}$ and Wild type mice after injury with βcatenin and troponin antibodies shows minimal expression of βcatenin over the epicardium (arrowhead) in Wt-1/Cre/βcatenin$^{fl/fl}$ mice but preserved βcatenin expression over troponin positive myocytes (arrow) (**F**) X gal staining of Wt-1 derived cells from adult Wt-1Cre/R26R^{lacZ} mouse heart. Western Blot shows βcatenin expression in Wt-1 derived cells isolated from Wt-1Cre/βcatenin$^{fl/fl}$ mice; Densitometry on right; (*P<0.05; mean±S.E.M, n=3) (**G**) TTC and Evans blue staining for determining infarct area (IA) to area at risk (AAR) ratios between βcatenin+/+ and Wt-1 βcatenin KO mice demonstrates no significant differences in IA/AAR ratios measured 24 hours after ischemic injury. (Arrows point to pale area that indicate infarct area, red area indicates viable tissue and blue area (stained with Evans Blue) indicates the area not at risk; n.s: not significant, n=3, Scale bar: A, 100 μm; C-E, 50 μm.

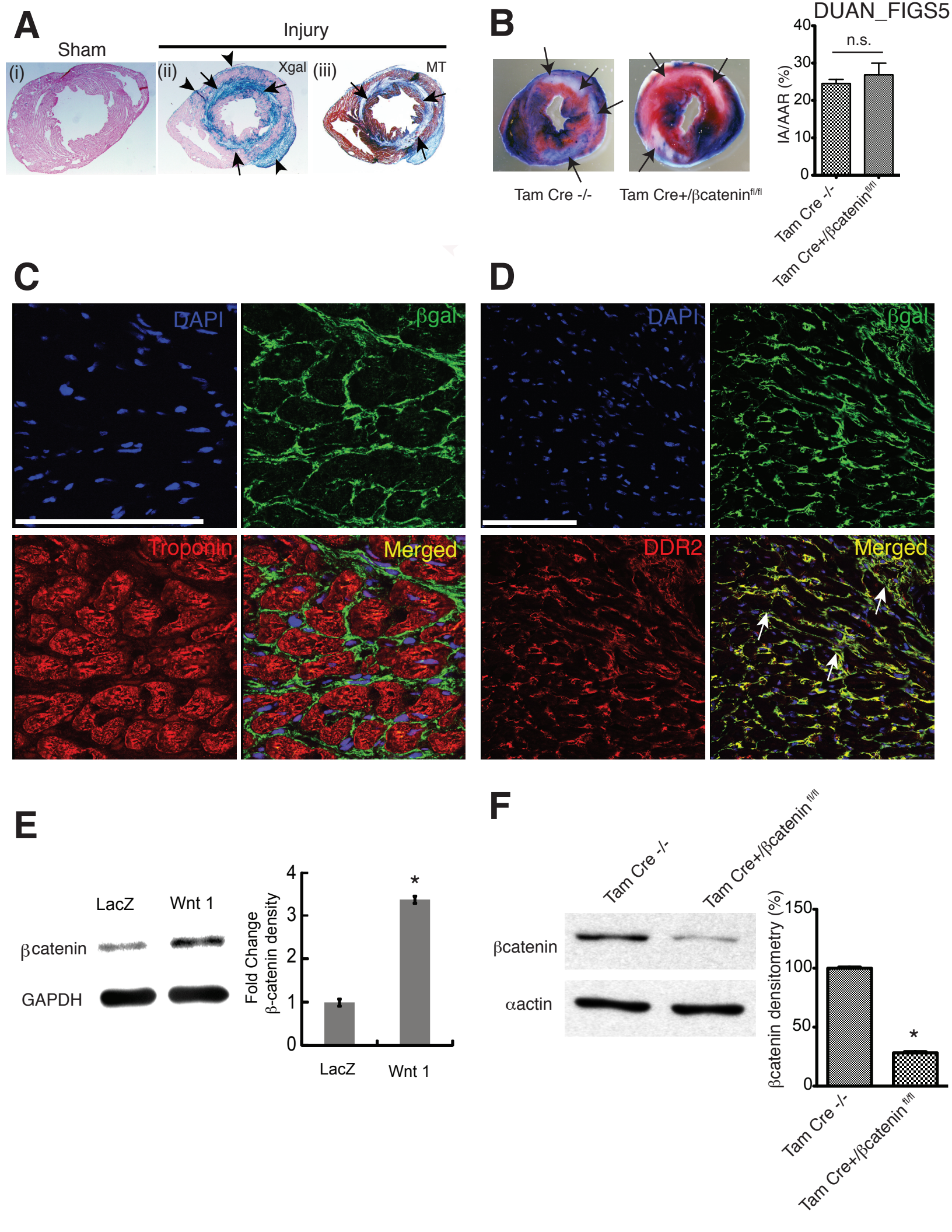

A
Sham
Injury
(i)
(ii)
Xgal
(iii)
MT
B
DUAN_FIGS5
n.s.
IA/AAR (%)
40
30
20
10
0
Tam Cre -/-
Tam Cre+/βcatenin fl/fl
Tam Cre -/-
Tam Cre+/βcatenin fl/fl
C
DAPI
βgal
Troponin
Merged
D
DAPI
βgal
DDR2
Merged
E
LacZ
Wnt 1
βcatenin
GAPDH
Fold Change β-catenin density
4
3
2
1
0
*
LacZ
Wnt 1
F
Tam Cre -/-
Tam Cre+/βcatenin fl/fl
βcatenin
αactin
βcatenin densitometry (%)
150
100
50
0
*
Tam Cre -/-
Tam Cre+/βcatenin fl/fl

FIGURE S5. Cardiac fibroblasts in Col1a2CreER(T)/R26R^{lacZ} mice and activation of canonical Wnt signaling in cardiac fibroblasts

Xgal staining of hearts of Col1a2CreER(T)/R26R^{lacZ} mice following administration of tamoxifen (**A**) Xgal staining of (i)heart section 11 days after sham injury (ii)heart section 11 days after ischemia injury and (iii)Masson trichrome staining of same (arrows point to area of injury and arrowheads to the epicardium/subepicardium). (**B**) TTC and Evans blue staining in tamoxifen injected Cre negative and positive mice to determine infarct area to area at risk (IA/AAR) ratios **(C-D)** Double immunostaining of area of injury with **(C)** Troponin and βgal antibodies shows lack of co-localization of βgal and troponin expression, with βgal expression observed on the myocyte interstitium **(D)** βgal and DDR2 antibodies shows abundance of cells expressing both markers (arrows) **(E)** Western blotting and corresponding densitometric analysis of cytoplasmic βcatenin levels 48 hours following Wnt1 lentiviral infection in cardiac fibroblasts (n=3,*$p<0.05$ compared to lacZ infected cardiac fibroblasts (**F**) Western Blot for βcatenin expression in cardiac fibroblasts isolated from Col1a2Cre/βcatenin$^{fl/fl}$ mice following 10 day tamoxifen injection. Tamoxifen injected Cre negative mice serve as controls. Densitometry shown on right ($p<0.05$, mean± S.E.M, n=3). Scale bar: 100 μm.

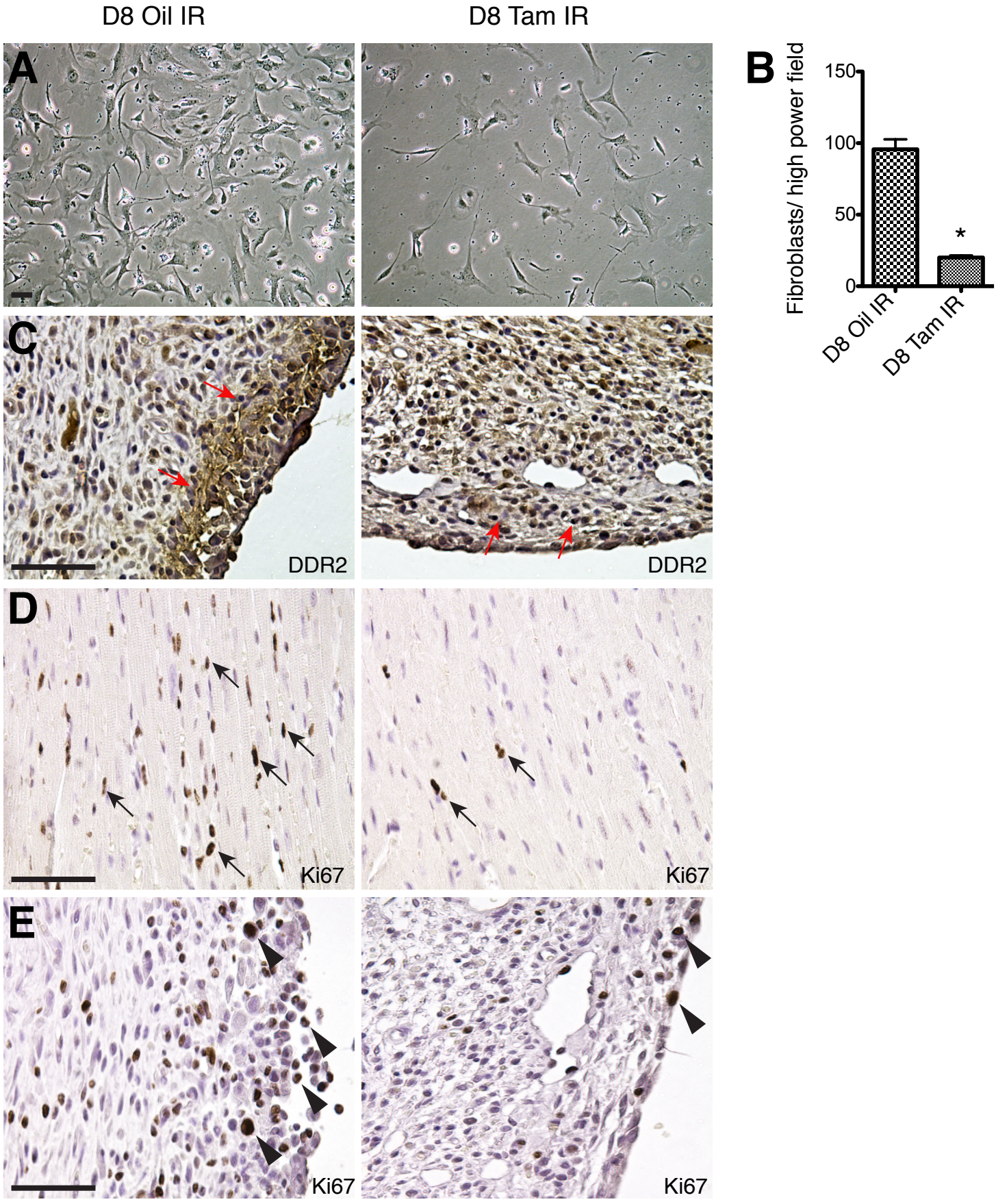

D8 Oil IR
D8 Tam IR
A
B
Fibroblasts/ high power field
150
100
50
0
*
D8 Oil IR
D8 Tam IR
C
DDR2
DDR2
D
Ki67
Ki67
E
Ki67
Ki67

FIGURE S6. Decreased fibroblast numbers in Col1a2CreER(T)/βcatenin$^{fl/fl}$ mice after cardiac injury

Fibroblasts isolated from (**A**) oil injected or tamoxifen injected Col1a2CreER(T)/ βcatenin$^{fl/fl}$ mice 8 days following cardiac injury demonstrates decreased fibroblast numbers in tamoxifen injected mice 48 hours after isolation. (**B**) Quantitation of fibroblast numbers/high power field 48 hours after isolation. (**C**) Immunohistochemistry for DDR2 on heart sections of oil or tamoxifen injected animals 8 days following injury; red arrows point to DDR2 expression in the epicardium/subepicardium (**D**) Immunohistochemistry for Ki67 on heart section of oil or tamoxifen injected animals 8 days following injury; arrows point to Ki67 positive cells close to the area of injury (**E**) Imunohistochemistry for Ki67 on heart sections of oil or tamoxifen injected animals 8 days following injury demonstrating increased Ki67 expression in the epicardial/ subepicardial region of hearts of oil injected animals (arrowheads) Scale bar: 50μm.

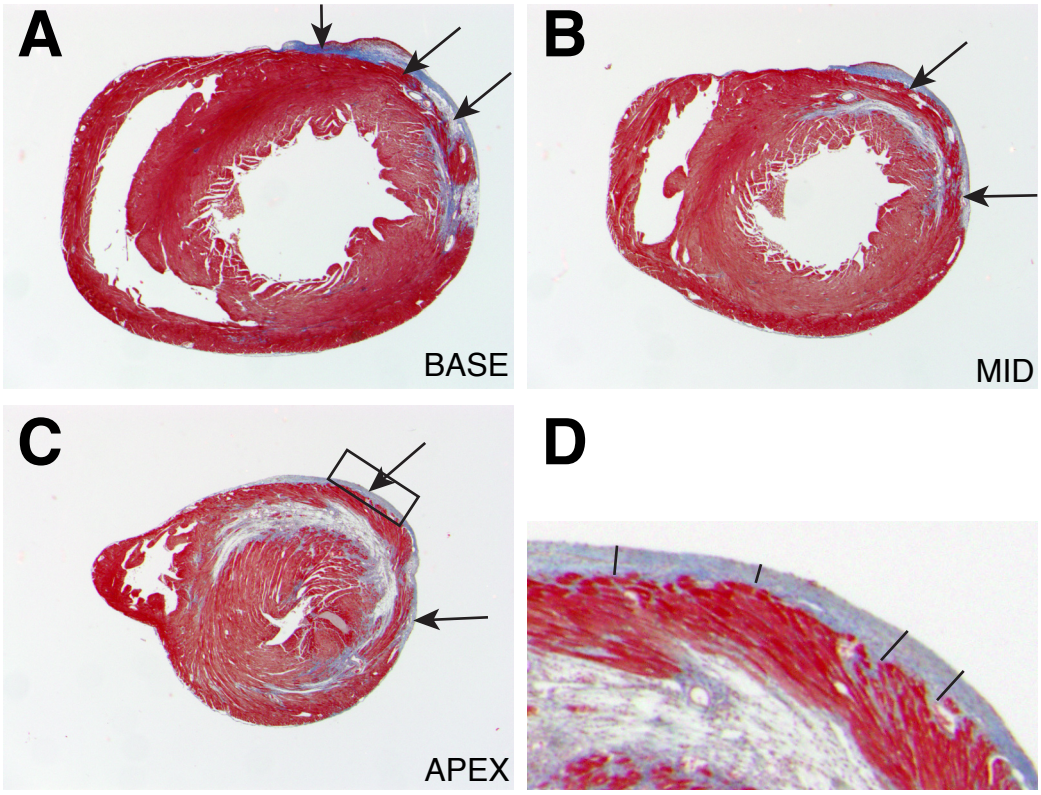


FIGURE S7. Measurement of epicardial thickness following Masson Trichrome staining The thickness of the epicardium was measured in sections with injury. Separate measurements were made at heart sections close to the base of the heart (**A**), mid ventricle (**B**) and apex (**C**). In each section, epicardial thickness was measured in 3-5 regions depending upon the circumferential thickening of the epicardium. Measurements were made by drawing a line perpendicular from the epi-myocyte border to the outer edge of the epicardium as shown by black lines in (**D**, boxed area highlighted in C). Arrows demonstrate thickening of the epicardium. All epicardial measurements taken at the base, mid and apex were averaged to determine the “mean” epicardial thickness.

Table S1. Echocardiographic parameters of Wt-1Cre:βcatenin$^{fl/fl}$ mice before and after ischemia reperfusion injury

Genotype	Heart rate	PW (mm)	IVS (mm)	LVEDD (mm)	LVESD (mm)	FS (%)	LVESV (μl)	LVEDV (μl)
βcatenin +/+ Pre IR	630±20	1.36± 0.32	1.53± 0.19	1.92±0.33	0.75±0.31	64±9.8	1.3±1.5	12.1±5.6
WT1:βcatenin KO Pre IR	664±58	1.36± 0.22	1.46± 0.15	2.24±0.29	1.00±0.20	56±4.3	2.2±1.1	17.4±5.5
βcatenin +/+ D8 Post IR	737±34	1.39± 0.33	1.71± 0.32	2.30±0.35	1.03±0.28	58±6.8	2.6±1.9	18.8±6.8
WT1:βcatenin KO D8 Post IR	693±31	1.30± 0.18	1.65± 0.28	2.72±0.29	1.47±0.28	46±6.7*	6.1±2.9*	28.1±7.6*

All data represent means±S.D. * All parameters were statistically significant when compared to βcatenin +/+ mice D8 Post IR. All echocardiographic parameters of Wt-1:βcatenin KO mice Pre IR were statistically non-significant when compared to βcatenin +/+ mice Pre IR. LVEDD and LVESD refer to end diastolic and systolic dimensions. PW and IVS refer to posterior left ventricular wall and inter-ventricular septum thickness. P value was computed using a two way Anova grouped analysis with Bonferroni's post test. (n= 6 animals/group). Heart rate (mean±S.D.) refers to mean heart rate during acquisition of images.

Table S2. Echocardiographic parameters of Col1a2Cre:βcatenin$^{fl/fl}$ mice before and after ischemia reperfusion injury

Genotype	Heart rate	PW (mm)	IVS (mm)	LVEDD (mm)	LVESD (mm)	FS (%)	LVESV (μl)	LVEDV (μl)
Oil Pre IR	567±49	1.49± 0.16	1.59± 0.20	2.54±0.51	1.09±0.40	58±8.1	3.3±2.8	24.7±12.1
D8 Oil IR	638±32	1.20± 0.2	1.38± 0.21	2.67±0.5	1.40±0.46	48±8.5	6.2±5.3	27.9±13.3
Tam Cre -/- Pre IR	630±75	1.77± 0.11	1.66± 0.16	2.67±0.46	1.15±0.20	59±4.7	2.1±0.97	23±8.2
D8 Tam Cre -/- IR	665±35	0.99± 0.26	1.35± 0.09	2.8±0.21	1.42±0.16	48±12.6	6.8±4.78	30.7±5.0
Tam Pre IR	558±29	1.48± 0.19	1.48± 0.17	2.47±0.24	0.84±0.13	66±4.14	1.4±0.69	21.6±4.9
D8 Tam IR	610±61	0.89± 0.28	1.14± 0.49	2.90±0.57	2.01±0.91	33±18.5*	29.9±2.8*	47.6±1.5*

All data represent mean±S.D. * Statistically significant when compared to D8 Tam Cre -/- IR and D8 Oil IR mice groups. P value computed with one way Anova with Bonferroni's post test analysis (n=15 animals/group). Heart rate refers to mean heart rate during acquisition of images. LVEDD and LVESD refer to end diastolic and systolic dimensions. PW and IVS refer to posterior left ventricular wall and inter-ventricular septum thickness.