Supplemental Materal

Supplemental Methods

In vitro immunofluorescence

Cultures of rat ventricular myocytes were fixed and prepared for immunofluorescence analysis as previously described.[1,2] Primary antibodies included mouse monoclonal anti-Cx43 (Millipore MAB3067, 1:200), anti-N-cadherin (SIGMA C1821, 1:400), anti-plakoglobin (SIGMA P8087, 1:800), and anti-GSK3β (Cell Signaling Technology, 27C10, 9315S, 1:100) antibodies, and a rabbit polyclonal anti-phospho-NFκB p65 (Cell Signaling Technology, 3033S, 1:200) antibody. Indocarbocyanine (Cy3)-conjugated goat anti-mouse IgG (Jackson Immunolabs, 1:400) or Cy3-conjugated goat anti-rabbit IgG (Jackson Immunolabs, 1:400) were used as secondary antibodies, and DAPI was used to visualize nuclei. Immunostained preparations were visualized by confocal microscopy and changes in the spatial distribution of signals were assessed qualitatively.

iPSC-CM monolayers for immunostaining were rinsed with PBS (pH 7.4) before fixation with ice cold 100% methanol. Samples were rinsed again in PBS, and permeabilized with 0.2% Triton X-100 in PBS. Samples were again rinsed in PBS, and then incubated in 10% normal goat serum (ThermoFisher Scientific, #50062Z). Primary antibodies included a rabbit polyclonal anti-NF-kB p65 antibody (Abcam, ab86299; 1:250), and mouse monoclonal anti-cardiac troponin I (US Biological, T8665-13F; 1:250) and anti-plakoglobin (Sigma, 8087; 1:150) antibodies. Secondary antibodies included goat anti-rabbit Alexa Fluor 568 (Invitrogen, A11011; 1:250) and goat anti-mouse Alexa Fluor 488 (ThermoFisher Scientific, A11001; 1:250).

In vitro apoptosis assays

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) was performed

on neonatal rat myocyte cultures according to the manufacturer's protocol (Millipore, S7110). The number of TUNEL-positive nuclei was counted in 5 randomly selected high-power fields in each culture well and expressed as a per cent of total nuclei visualized with DAPI.

In vitro cytokine assays

Culture media from cells expressing *JUP*^{2157del2} in the presence or absence of Bay11-7082 (5 μM, 24 hours) were collected after 96 hours in culture, mixed with a cocktail of biotinylated detection antibodies, and incubated with nitrocellulose membranes spotted in duplicate with control and capture antibodies (R&D Systems). Chemiluminescent signal produced at each spot corresponded to the amount of bound cytokine. Conditioned media from non-transfected cultures were assayed for cytokine expression as controls. The cytokine array specifically detected rat cytokines whereas cells were grown in media containing neonatal bovine serum. Thus, cross contamination was presumably insignificant.

Quantification of myocardial fibrosis

Image J software was used to trace the profile of the ventricular myocardium and all fibrotic areas (blue) in sections stained with Masson trichrome. The percent fibrosis was calculated as the sum of all fibrotic areas divided by total ventricular myocardium area X 100 in each slide.

Mouse myocardial tissue immunofluorescence and immunoperoxidase

Immunofluorescence staining was used to characterize the distribution of selected proteins at cell-cell junctions as previously reported.[1,2] Primary antibodies included rabbit polyclonal anti-Cx43 (SIGMA C6219, 1:200), anti-N-cadherin (SIGMA C3678, 1:400), anti-plakoglobin (Thermo Fisher Scientific PA5-17320, 1:500), anti-GSK3β (Cell Signaling Technology 27C10, 9315S, 1:80) and anti-SAP97 (Abcam, ab134156, 1:200). Cy3-conjugated

goat anti-rabbit IgG (Jackson Immunolabs, 1:400) was used as secondary antibody. Immunostained preparations were visualized by confocal microscopy. Additional sections prepared with Masson trichrome and hematoxylin & eosin stains were examined by light microscopy.

Immunoperoxidase staining was used to detect expression of selected cytokines in sections of mouse myocardium as previously reported.[3] Primary antibodies included rabbit polyclonal anti-TNFα (AbCam ab34674, 1:50 dilution), anti-IL-1β (AbCam ab9722, 1:500) and anti-MCP-1α (Novus Biologicals NBP2-41209, 1:250). After standard development in diaminobenzidine, sections were counterstained with hematoxylin and examined qualitatively by light microscopy. In each experiment, control, vehicle-treated and drug-treated samples were batched to ensure identical staining and signal-generating conditions.

Immunoperoxidase staining using conventional techniques was also used to detect T-cells and macrophages in myocardial tissue sections from *Dsg2*$^{mut/mut}$ mice. T-cells were identified using an anti-CD3 antibody (Abcam ab16669, 1:200 dilution) and macrophages were marked with an anti-CD68 antibody (Abcam ab127055, 1:400). After standard development in diaminobenzidine, sections were counterstained with hematoxylin and examined qualitatively by light microscopy.

phospho-RelA/p65 western blots

Mouse ventricular myocardium was lysed in RIPA buffer containing 1:100 phosphatase and proteinase inhibitor cocktails (Sigma-Aldrich), and protein content was quantified using standard Pierce BCA protein assay kits (Life Technologies). Aliquots containing 40 µg protein were separated on 4-12% BisTris gels (NuPage, Invitrogen) with 1X MOPS Running Buffer (Invitrogen) at 200V constant. Gels were transferred to nitrocellulose membranes and blocked

for 1 hour at room temperature in 1X PBS containing 5% nonfat milk and 0.1% Tween-20. The resultant blots were probed with primary antibodies overnight at 4°C in blocking buffer. Primary antibodies included rabbit polyclonal anti-phospho-RelA/p65 (at Ser536) (Abcam, 86200; 1:1000), mouse monoclonal anti-total-RelA/p65 (Cell Signaling, 5174S; 1:5000) and rabbit monoclonal anti-GAPDH (Cell Signaling, 5174S; 1:5000) antibodies. After being incubated with primary antibodies, blots were washed three times (1X PBS with 0.1% Tween-20) and probed with species-specific IRDye secondary antibodies (Li-Cor, IRDye 800CW or IRDye 680RD, 1:5,000) for 1 hour in blocking buffer at room temperature. They were then washed three times (1X PBS with 0.1% Tween-20), and examined using the LI-COR Odyssey imaging system.

Supplemental Table 1: Fold-changes in cytokine expression in the hearts of *Dsg2*$^{mut/mut}$ mice and its attenuation by Bay 11-7082.

Target	*Dsg2*$^{mut/mut}$ vs WT		*Dsg2*$^{mut/mut}$ (Bay11-7082) vs WT		*Dsg2*$^{mut/mut}$ (Bay11-7082) vs *Dsg2*$^{mut/mut}$	
	Fold Change	P-Values	Fold Change	P-Values	Fold Change	P-Values
Adiponectin	<1	0.51	<1	0.13	<1	0.60
Amphiregulin	<1	0.18	<1	0.05*	<1	0.86
Angiopoietin-1	<1	0.59	<1	0.27	<1	0.82
Angiopoietin-2	<1	0.73	<1	0.82	1-2	0.92
Angiopoietin-like 3	<1	0.25	<1	0.25	<1	0.78
BAFF/BLyS/TNFSF13B	<1	0.27	<1	0.13	<1	0.16
C1qR1/CD93	1-2	0.99	<1	0.30	<1	0.25
CCL2/JE/MCP-1	<1	0.22	<1	0.17	<1	0.32
CCL3/CCL4/MIP-1a/B	<1	0.18	<1	0.15	<1	0.50
CCL5/RANTES	<1	0.29	<1	0.09	<1	0.75
CCL6/C10	<1	0.91	<1	0.24	<1	0.22
Eotaxin (CCL11)	<1	0.59	<1	0.57	<1	0.90
MCP-5 (CCL12)	<1	0.30	<1	0.31	<1	0.80
CCL17/TARC	<1	0.87	<1	0.20	<1	0.26
CCL19/MIP-3B	<1	0.98	<1	0.36	<1	0.33
CCL20/MIP-3a	1-2	0.75	<1	0.32	<1	0.23
CCL21/6Ckine	1-2	0.22	<1	0.50	<1	0.03*
CCL22/MDC	<1	0.21	<1	0.12	<1	0.88
CD14	<1	0.16	<1	0.09	1-2	0.88
CD40/TNFRSF5	<1	0.50	<1	0.15	<1	0.39
CD160	1-2	0.95	<1	0.22	<1	0.19
Chemerin	<1	0.44	<1	0.21	<1	0.40
Chitinase 3-like 1	<1	0.78	<1	0.37	<1	0.37
Coagulation Factor III/Tissue Factor	<1	0.42	<1	0.08	<1	0.36
Complement Component C5/C5a	1-2	0.92	<1	0.36	<1	0.25
Complement Factor D	2-4	0.12	<1	0.32	<1	0.02*
C-Reactive Protein/CRP	1-2	0.54	<1	0.20	<1	0.09
CX3CL1/Fractalkine	<1	0.38	<1	0.50	<1	0.87
KC (CXCL1)	<1	0.53	<1	0.25	<1	0.45
MIP-2 (CXCL2)	<1	0.27	<1	0.15	<1	0.89
CXCL9/MIG	1-2	0.82	<1	0.11	<1	0.13
IP-10 (CXL10/CRG-2)	1-2	0.22	<1	0.63	<1	0.23
I-TAC (CXCL11)	1-2	0.51	<1	0.07	<1	0.09
CXCL13/BLC/BCA-1	6-10	0.20	2-4	0.70	<1	0.35
CXCL16	1-2	0.46	<1	0.32	<1	0.02*
Cystatin C	1-2	0.31	<1	0.29	<1	0.10
DKK-1	2-4	0.25	<1	0.95	<1	0.26
DPPIV/CD26	4-6	0.05*	1-2	0.53	<1	0.03*
EGF	2-4	0.23	1-2	0.99	<1	0.17
Endoglin/CD105	<1	0.86	<1	0.09	<1	0.02*
Endostatin	1-2	0.68	<1	0.39	<1	0.13
Fetuin A/AHSG	1-2	0.93	<1	0.08	<1	0.04*
FGF acidic	1-2	0.44	<1	0.73	<1	0.01*
FGF-21	1-2	0.82	<1	0.11	<1	0.13
Flt-3 Ligand	2-4	0.32	<1	0.63	<1	0.09
Gas 6	1-2	0.18	<1	0.74	<1	0.04*
G-CSF	2-4	0.01*	1-2	0.83	<1	0.16
GDF-15	1-2	0.59	<1	0.19	<1	0.92
GM-CSF	<1	0.94	<1	0.20	<1	0.67
HGF	>10	0.05*	4-6	0.28	<1	0.05*
sICAM-1 (CD54)	1-2	0.04*	1-2	0.97	<1	0.09
IFN-γ	4-6	0.09	1-2	0.82	<1	0.05*
IGFBP-1	2-4	0.14	1-2	0.66	<1	0.72
IGFBP-2	2-4	0.35	<1	0.75	<1	0.10
IGFBP-3	1-2	0.44	<1	0.18	<1	0.02*
IGFBP-5	1-2	0.34	<1	0.40	<1	0.09
IGFBP-6	2-4	0.31	<1	0.37	<1	0.05*
IL-1a (IL-1F1)	2-4	0.14	<1	0.92	<1	0.13
IL-1β (IL-1F2)	>10	0.01*	6-10	0.31	<1	0.27
IL-1ra (IL-1F3)	4-6	0.001*	1-2	0.50	<1	0.02*
IL-2	<1	0.53	<1	0.20	<1	0.37
IL-3	<1	0.53	<1	0.20	<1	0.37
IL-4	1-2	0.25	<1	0.35	<1	0.12
IL-5	<1	0.66	<1	0.34	<1	0.95
IL-6	<1	0.53	<1	0.25	<1	0.45
IL-7	1-2	0.70	<1	0.65	<1	0.31
IL-10	1-2	0.14	<1	0.67	<1	0.08
IL-11	1-2	0.24	<1	0.81	<1	0.21
IL-12 p40	4-6	0.16	1-2	0.57	<1	0.16
IL-13	4-6	0.11	1-2	0.44	<1	0.20
IL-15	6-10	0.004*	2-4	0.31	<1	0.31
IL-17a	<1	0.53	<1	0.20	<1	0.37
IL-22	<1	0.53	<1	0.20	<1	0.37
IL-23	<1	0.92	<1	0.20	<1	0.85
IL-27 p28	2-4	0.16	<1	0.49	<1	0.04*
IL-28A/B	1-2	0.29	1-2	0.89	<1	0.63
IL-33	2-4	0.07	2-4	0.18	<1	0.29
LDL R	<1	0.83	1-2	0.95	1-2	0.76
Leptin	2-4	0.04*	1-2	0.62	<1	0.03*
LIF	1-2	0.47	<1	0.93	<1	0.18
Lipocalin-2/NGAL	<1	0.96	<1	0.40	<1	0.83
LIX	>10	0.003*	>10	0.04*	<1	0.003*
M-CSF	>10	0.03*	>10	0.003*	1-2	0.60
MMP-2	4-6	0.01*	4-6	0.02*	1-2	0.79
MMP-3	<1	0.81	<1	0.42	1-2	0.64
MMP-9	<1	0.82	<1	0.20	<1	0.33
Myeloperoxidase	4-6	0.17	2-4	0.12	<1	0.14
Osteopontin (OPN)	>10	0.01*	4-6	0.27	<1	0.0009*
Osteoprotegerin/TNFRSF11B	<1	0.53	<1	0.25	<1	0.45
PD-EGF/Thymidine phosphorylase	<1	0.53	<1	0.20	<1	0.37
PDGF-BB	2-4	0.39	1-2	0.96	<1	0.28
Pentraxin 2/SAP	4-6	0.07	1-2	0.62	<1	0.07
Pentraxin 3/TSG-14	<1	0.96	<1	0.09	<1	0.15
Periostin/OSF-2	<1	0.61	<1	0.34	<1	0.84
Pref-1/DLK-1/FA1	6-10	0.03*	6-10	0.003*	<1	0.76
Proliferin	6-10	0.01*	6-10	0.03*	<1	0.86
Proprotein Convertase 9/PCSK9	<1	0.86	<1	0.39	1-2	0.82
RAGE	<1	0.92	<1	0.23	<1	0.65
RBP4	1-2	0.10	1-2	0.18	<1	0.79
Reg3G	6-10	0.04*	6-10	0.02*	<1	0.93
Resistin	2-4	0.01*	2-4	0.27	<1	0.09
E-Selectin/CD62E	<1	0.71	<1	0.15	<1	0.13
P-Selectin/CD62P	>10	0.09	>10	0.21	<1	0.14
Serpin E1/PAI-1	>10	0.07	6-10	0.03*	<1	0.33
Serpin F1/PEDF	<1	0.96	<1	0.68	<1	0.70
Thrombopoietin	<1	0.94	<1	0.34	1-2	0.93
TIM-1/KIM-1/HAVCR	<1	0.59	<1	0.16	1-2	0.46
TNF-α	2-4	0.66	1-2	0.61	<1	0.85
VCAM-1/CD106	4-6	0.35	6-10	0.01*	1-2	0.39
VEGF	<1	0.55	<1	0.71	1-2	0.96
WISP-1/CCN4	<1	0.53	<1	0.30	<1	0.51

Fold Change
<1
1-2
2-4
4-6
6-10
>10
>20

Supplemental Table 1. Data are presented as fold-change; P values are shown for vehicle-treated *Dsg2*$^{mut/mut}$ (columns 1 and 2) and Bay11-7082 treated *Dsg2*$^{mut/mut}$ mice (columns 3 and 4) vs. vehicle-treated WT mice; and Bay11-7082 treated *Dsg2*$^{mut/mut}$ mice vs. vehicle-treated *Dsg2*$^{mut/mut}$ mice (columns 5 and 6). P values were determined using one-way ANOVA with Tukey's multiple comparisons test; * $P \leq 0.05$ for comparisons between cohorts within columns (e.g., *Dsg2*$^{mut/mut}$ vs. WT); n=5 for vehicle-treated WT and *Dsg2*$^{mut/mut}$ mice; n=10 for Bay11-7082-treated *Dsg2*$^{mut/mut}$ mice.

Supplemental Table 2. Fold-change in cytokine expression in hiPSC-CMs and its attenuation by Bay 11-7082.

Target	*PKP2* vs Control		*PKP2* (Bay11-7082) vs Control		*PKP2* (Bay11-7082) vs *PKP2*		Control (Bay11-7082) vs Control	
	Fold Change	P-Values	Fold Change	P-Values	Fold Change	P-Values	Fold Change	P-Values
Adiponectin	>5	0.05*	>5	0.47	<2	0.04*	3-5	0.47
Apolipoprotein A-1	<2	0.38	2-3	0.41	<2	0.97	<2	0.17
Angiogenin	>5	0.04*	>5	0.20	<2	0.05*	<2	0.38
Angiopoietin-1	3-5	0.36	2-3	0.33	<2	0.31	<2	0.07
Angiopoietin-2	2-3	0.41	2-3	0.33	<2	0.56	<2	0.03*
BAFF/BLyS/TNFSF13B	>5	0.40	2-3	0.33	<2	0.35	<2	0.13
BDNF	>5	0.38	>5	0.28	<2	0.43	<2	0.11
Complement Component C5/C5a	3-5	0.38	<2	0.93	<2	0.29	<2	0.13
CD14	3-5	0.18	<2	0.47	<2	0.09	<2	0.16
CD30	<2	0.82	<2	0.31	<2	0.12	<2	0.18
CD40 Ligand	2-3	0.08	<2	0.86	<2	0.01*	<2	0.18
Chitinase 3-like 1	2-3	0.21	2-3	0.30	<2	0.43	<2	0.14
Complement Factor D	2-3	0.01*	2-3	0.31	<2	0.13	<2	0.003*
C-Reactive Protein/CRP	3-5	0.38	3-5	0.24	<2	0.38	<2	0.001*
Cripto-1	>5	0.35	2-3	0.40	<2	0.29	<2	0.14
Cystatin C	>5	0.33	>5	0.28	<2	0.33	<2	0.15
DKK-1	3-5	0.26	3-5	0.27	<2	0.27	<2	0.17
DPPIV/CD26	>15	0.35	>5	0.37	<2	0.33	<2	0.01*
EGF	3-5	0.11	<2	0.92	<2	0.05*	<2	0.13
Emmprin (CD147)	<2	0.04*	<2	0.02*	<2	0.001*	<2	0.05*
LIX (Cxcl5)	2-3	0.18	<2	0.65	<2	0.05*	<2	0.01*
Endoglin/CD105	<2	0.36	<2	0.42	<2	0.55	<2	0.03*
Fas Ligand	2-3	0.45	3-5	0.33	<2	0.79	<2	0.02*
FGF basic	2-3	0.35	2-3	0.37	<2	0.32	<2	0.01*
FGF-7	2-3	0.33	<2	0.78	<2	0.18	<2	0.03*
FGF-19	<2	0.18	<2	0.95	<2	0.11	<2	0.03*
Flt-3 Ligand	>5	0.24	2-3	0.41	<2	0.20	<2	0.13
G-CSF	3-5	0.23	<2	0.95	<2	0.13	<2	0.14
GDF-15	>5	0.05*	2-3	0.60	<2	0.03*	<2	0.14
GM-CSF	2-3	0.23	<2	0.73	<2	0.09	<2	0.19
GROα (Cxcl1)	2-3	0.16	<2	0.94	<2	0.07	<2	0.13
GH, Somatotropin	2-3	0.19	<2	0.72	<2	0.09	<2	0.14
HGF	2-3	0.20	2-3	0.33	<2	0.22	<2	0.0004*
ICAM-1 (CD54)	2-3	0.28	<2	0.49	<2	0.24	<2	0.004*
IFN-γ	3-5	0.32	>5	0.31	2-3	0.52	<2	0.003*
IGFBP-2	3-5	0.09	>5	0.15	2-3	0.27	<2	0.03*
IGFBP-3	3-5	0.20	<2	0.78	<2	0.10	<2	0.11
IL-1α (IL-1F1)	>5	0.23	>5	0.27	<2	0.31	<2	0.08
IL-1β (IL-1F2)	2-3	0.05*	<2	0.60	<2	0.01*	<2	0.07
IL-1ra (IL-1F3)	3-5	0.22	<2	0.53	<2	0.12	<2	0.17
IL-2	2-3	0.30	<2	0.57	<2	0.15	<2	0.15
IL-3	3-5	0.26	<2	0.81	<2	0.18	<2	0.12
IL-4	2-3	0.17	2-3	0.63	<2	0.16	<2	0.15
IL-5	>15	0.33	>15	0.24	<2	0.33	<2	0.15
IL-6	2-3	0.27	<2	0.52	<2	0.22	<2	0.01*
IL-8	<2	0.52	<2	0.33	<2	0.20	<2	0.004*
IL-10	>5	0.34	3-5	0.31	<2	0.31	<2	0.001*
IL-11	2-3	0.25	<2	0.48	<2	0.18	<2	0.01*
IL-12 p70	3-5	0.26	3-5	0.31	<2	0.23	<2	0.07
IL-13	>5	0.25	2-3	0.46	<2	0.20	<2	0.13
IL-15	3-5	0.04*	<2	0.97	<2	0.01*	<2	0.13
IL-16	3-5	0.19	<2	0.74	<2	0.10	<2	0.13
IL-17a	2-3	0.17	<2	0.90	<2	0.04*	<2	0.17
IL-18	>5	0.35	2-3	0.64	<2	0.30	<2	0.13
IL-19	2-3	0.20	<2	0.94	<2	0.07	<2	0.14
IL-22	2-3	0.18	<2	0.85	<2	0.07	<2	0.04*
IL-23	3-5	0.31	<2	0.62	<2	0.24	<2	0.06
IL-24	3-5	0.25	2-3	0.34	<2	0.21	<2	0.01*
IL-27	3-5	0.29	2-3	0.30	<2	0.27	<2	0.01*
IL-31	3-5	0.33	2-3	0.31	<2	0.28	<2	0.01*
IL-32	>5	0.22	2-3	0.28	<2	0.19	<2	0.06
IL-33	>5	0.11	2-3	0.36	<2	0.08	<2	0.14
IL-34	3-5	0.11	<2	0.75	<2	0.06	<2	0.13
IP-10 (Cxcl10)	2-3	0.16	<2	0.97	<2	0.07	<2	0.16
I-TAC (Cxcl11)	>5	0.36	<2	0.86	<2	0.30	<2	0.16
Kallikrein-3 (PSA)	>5	0.36	>5	0.24	<2	0.34	<2	0.15
Leptin	<2	0.44	<2	0.96	<2	0.30	<2	0.16
LIF	2-3	0.23	<2	0.82	<2	0.09	<2	0.14
Lipocalin-2/NGAL	2-3	0.35	2-3	0.37	<2	0.32	<2	0.05*
MCP1	2-3	0.31	2-3	0.37	<2	0.30	<2	0.01*
MCP3	3-5	0.36	3-5	0.31	<2	0.32	<2	0.02*
M-CSF	3-5	0.35	>5	0.31	<2	0.40	<2	0.01*
MIF	>5	0.01*	3-5	0.08	<2	0.01*	<2	0.10
MIG (Cxcl9)	2-3	0.13	<2	0.75	<2	0.05*	<2	0.08
MIP-1α/MIP-1β	2-3	0.25	<2	0.12	<2	0.05*	<2	0.03*
MIP-3α	2-3	0.26	<2	0.89	<2	0.16	<2	0.09
MIP-3β	2-3	0.33	<2	0.91	<2	0.16	<2	0.18
MMP-9	3-5	0.37	2-3	0.63	<2	0.31	<2	0.20
Myeloperoxidase	3-5	0.34	<2	0.46	<2	0.27	<2	0.25
Osteopontin	<2	0.37	<2	0.35	<2	0.10	<2	0.01*
PDGF-AA	<2	0.38	<2	0.51	<2	0.13	<2	0.02*
PDGF-AB/BB	2-3	0.41	<2	0.19	<2	0.25	<2	0.00001*
Pentraxin 3/TSG-14	2-3	0.28	3-5	0.36	<2	0.81	<2	0.01*
PF4 (Cxcl4)	3-5	0.32	2-3	0.36	<2	0.28	<2	0.02*
RAGE	3-5	0.21	<2	0.80	<2	0.13	<2	0.06
RANTES	3-5	0.03*	<2	0.57	<2	0.01*	<2	0.08
RBP-4	<2	0.43	<2	0.44	<2	0.12	<2	0.03*
Relaxin-2	2-3	0.35	<2	0.94	<2	0.21	<2	0.02*
Resistin	<2	0.42	<2	0.47	<2	0.16	<2	0.03*
SDF-1α (Cxcl12)	<2	0.77	<2	0.63	<2	0.35	<2	0.16
Serpin E1/PAI-1	<2	0.25	<2	0.27	<2	0.07	<2	0.01*
SHBG (ABP)	2-3	0.22	<2	0.88	<2	0.02*	<2	0.20
ST2 (IL-1 R4)	2-3	0.26	<2	0.89	<2	0.13	<2	0.23
TARC (CCL17)	2-3	0.44	<2	0.65	<2	0.36	<2	0.18
TFF3	>5	0.29	<2	0.44	<2	0.24	<2	0.17
TfR (CD71)	>5	0.28	>5	0.23	<2	0.29	<2	0.16
TGF-α	>5	0.13	<2	0.60	<2	0.09	<2	0.28
Thrombospondin-1	>5	0.16	>5	0.25	<2	0.14	<2	0.20
TNF-α	2-3	0.12	<2	0.74	<2	0.03*	<2	0.13
uPAR	2-3	0.21	<2	0.89	<2	0.11	<2	0.15
VEGF	2-3	0.41	<2	0.98	<2	0.25	<2	0.12
Vitamin D BP	<2	0.50	<2	0.76	<2	0.36	<2	0.13
CD31 (PECAM-1)	<2	0.54	<2	0.80	<2	0.27	<2	0.20
TIM-3	3-5	0.31	<2	0.96	<2	0.20	<2	0.08
VCAM-1/CD106	2-3	0.11	<2	0.80	<2	0.05*	<2	0.08

Fold Change
<2
2-3
3-5
>5
>15

Supplemental Table 2. Data are presented as fold-change; P values are shown for untreated *PKP2* hiPSC-CMs vs. untreated control hiPSC-CMs (columns 1 and 2); Bay 11-7082-treated *PKP2* hiPSC-CMs vs. untreated control hiPSC-CMs (columns 3 and 4); Bay 11-7082-treated *PKP2* hiPSC-CMs vs. untreated *PKP2* hiPSC-CMs (columns 5 and 6); and Bay 11-7082-treated control hiPSC-CMs vs. untreated control hiPSC-CMs (columns 7 and 8). P values were determined by one-way ANOVA with Tukey's multiple comparisons test; * $P \leq 0.05$ for comparisons between cohorts within columns (e.g., *PKP2* vs. control); n=3 cell cultures for each cohort.

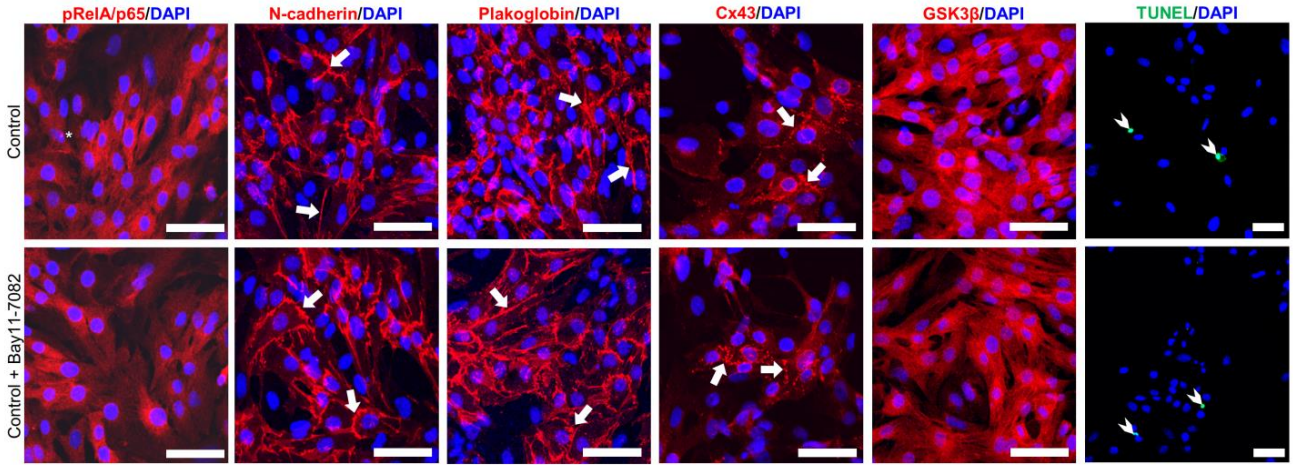
Supplemental Table 3. Fold-change in cytokine expression in hiPSC-CM culture media (supernatant) and its attenuation by Bay 11-7082.

Target	*PKP2* vs Control		*PKP2* (Bay11-7082) vs Control		*PKP2* (Bay11-7082) vs *PKP2*		Control (Bay11-7082) vs Control	
	Fold Change	P-Values	Fold Change	P-Values	Fold Change	P-Values	Fold Change	P-Values
Adiponectin	<2	0.42	<2	0.86	<2	0.43	<2	0.32
Apolipoprotein A-1	2-5	0.23	2-5	0.43	<2	0.91	<2	0.73
Angiogenin	<2	0.22	2-5	0.33	<2	0.59	<2	0.85
Angiopoietin-1	2-5	0.41	2-5	0.45	<2	0.92	<2	0.99
Angiopoietin-2	<2	0.47	2-5	0.33	<2	0.79	<2	0.97
BAFF/BLyS/TNFSF13B	2-5	0.38	<2	0.73	<2	0.55	<2	0.46
BDNF	2-5	0.40	<2	0.85	<2	0.48	<2	0.88
Complement Component C5/C5a	2-5	0.06	<2	0.12	<2	0.01*	<2	0.81
CD14	<2	0.27	<2	0.13	<2	0.01*	<2	0.75
CD30	<2	0.31	<2	0.17	<2	0.01*	<2	0.43
CD40 Ligand	2-5	0.08	<2	0.84	<2	0.34	2-5	0.50
Chitinase 3-like 1	5-10	0.23	2-5	0.62	<2	0.36	<2	0.57
Complement Factor D	<2	0.05*	<2	0.85	<2	0.76	<2	0.78
C-Reactive Protein/CRP	2-5	0.32	2-5	0.37	<2	0.96	<2	0.61
Cripto-1	2-5	0.32	2-5	0.48	<2	0.97	<2	0.97
Cystatin C	2-5	0.38	2-5	0.22	<2	0.50	<2	0.53
DKK-1	2-5	0.46	<2	0.81	<2	0.71	<2	0.50
DPPIV/CD26	5-10	0.38	2-5	0.54	<2	0.62	<2	0.35
EGF	2-5	0.21	<2	0.15	<2	0.01*	<2	0.98
Emmprin (CD147)	<2	0.44	<2	0.91	<2	0.43	<2	0.43
LIX (Cxcl5)	2-5	0.05*	<2	0.93	<2	0.22	<2	0.96
Endoglin/CD105	2-5	0.22	<2	0.75	<2	0.40	<2	0.95
Fas Ligand	5-10	0.21	2-5	0.30	<2	0.48	<2	0.56
FGF basic	<2	0.37	2-5	0.50	<2	0.82	<2	0.72
FGF-7	<2	0.45	2-5	0.44	<2	0.60	<2	0.71
FGF-19	<2	0.52	<2	0.84	<2	0.64	<2	0.36
Flt-3 Ligand	2-5	0.22	<2	0.93	<2	0.25	<2	0.68
G-CSF	>10	0.22	<2	0.37	<2	0.18	<2	0.71
GDF-15	5-10	0.32	>10	0.11	<2	0.55	<2	0.70
GM-CSF	2-5	0.24	<2	0.86	<2	0.23	<2	0.90
GROα (Cxcl1)	5-10	0.07	2-5	0.53	<2	0.05*	2-5	0.31
GH, Somatotropin	5-10	0.07	2-5	0.56	<2	0.26	2-5	0.44
HGF	2-5	0.31	<2	0.70	<2	0.72	<2	0.87
ICAM-1 (CD54)	2-5	0.07	2-5	0.62	<2	0.49	<2	0.98
IFN-γ	2-5	0.36	5-10	0.24	<2	0.91	<2	0.88
IGFBP-2	<2	0.43	2-5	0.25	2-5	0.21	<2	0.01*
IGFBP-3	2-5	0.05*	2-5	0.16	<2	0.95	<2	0.30
IL-1α (IL-1F1)	5-10	0.30	>10	0.23	<2	0.56	<2	0.73
IL-1β (IL-1F2)	2-5	0.15	<2	0.23	<2	0.0000003*	<2	0.94
IL-1ra (IL-1F3)	5-10	0.16	<2	0.13	<2	0.12	2-5	0.60
IL-2	2-5	0.14	<2	0.91	<2	0.40	<2	0.71
IL-3	2-5	0.06	<2	0.55	<2	0.01*	<2	0.95
IL-4	2-5	0.25	2-5	0.51	<2	0.78	<2	0.58
IL-5	5-10	0.13	>10	0.15	<2	0.37	2-5	0.46
IL-6	2-5	0.28	<2	0.60	<2	0.47	<2	0.84
IL-8	<2	0.40	<2	0.87	<2	0.50	<2	0.30
IL-10	2-5	0.33	2-5	0.44	<2	0.95	<2	0.95
IL-11	2-5	0.36	2-5	0.18	<2	0.95	<2	0.60
IL-12 p70	2-5	0.32	2-5	0.34	<2	0.83	<2	0.61
IL-13	2-5	0.21	<2	0.94	<2	0.30	<2	0.28
IL-15	2-5	0.24	<2	0.63	<2	0.12	<2	0.78
IL-16	2-5	0.18	<2	0.14	<2	0.09	<2	0.92
IL-17a	<2	0.33	<2	0.84	<2	0.03*	<2	0.48
IL-18	5-10	0.26	2-5	0.63	<2	0.39	<2	0.71
IL-19	5-10	0.20	2-5	0.56	<2	0.59	2-5	0.47
IL-22	2-5	0.18	2-5	0.60	<2	0.83	<2	0.66
IL-23	5-10	0.26	2-5	0.63	<2	0.44	<2	0.55
IL-24	2-5	0.30	2-5	0.46	<2	0.85	<2	0.85
IL-27	2-5	0.28	2-5	0.28	<2	0.82	<2	0.73
IL-31	5-10	0.28	2-5	0.36	<2	0.65	<2	0.70
IL-32	2-5	0.24	2-5	0.56	<2	0.65	<2	0.91
IL-33	2-5	0.15	<2	0.91	<2	0.22	<2	0.31
IL-34	2-5	0.15	<2	0.54	<2	0.10	<2	0.58
IP-10 (Cxcl10)	5-10	0.14	<2	0.46	<2	0.10	<2	0.84
I-TAC (Cxcl11)	>10	0.28	2-5	0.38	<2	0.34	<2	0.68
Kallikrein-3 (PSA)	5-10	0.31	2-5	0.26	<2	0.57	<2	0.90
Leptin	>10	0.04*	2-5	0.19	<2	0.05*	<2	0.59
LIF	>10	0.03*	2-5	0.19	<2	0.04*	<2	0.59
Lipocalin-2/NGAL	5-10	0.32	2-5	0.22	<2	0.74	<2	0.65
MCP1	2-5	0.51	5-10	0.03*	2-5	0.19	<2	0.47
MCP3	5-10	0.36	2-5	0.27	<2	0.71	<2	0.69
M-CSF	5-10	0.32	5-10	0.19	<2	0.91	2-5	0.58
MIF	<2	0.20	2-5	0.25	<2	0.43	<2	0.62
MIG (Cxcl9)	2-5	0.20	<2	0.76	<2	0.37	<2	0.96
MIP-1α/MIP-1β	5-10	0.26	<2	0.14	<2	0.18	<2	0.62
MIP-3α	5-10	0.15	<2	0.15	<2	0.11	<2	0.78
MIP-3β	2-5	0.24	<2	0.12	<2	0.15	<2	0.79
MMP-9	>10	0.26	<2	0.37	<2	0.25	5-10	0.45
Myeloperoxidase	>10	0.06	2-5	0.19	<2	0.11	<2	0.66
Osteopontin	2-5	0.02*	<2	0.13	<2	0.003*	<2	0.59
PDGF-AA	2-5	0.14	<2	0.51	<2	0.39	<2	0.41
PDGF-AB/BB	5-10	0.20	2-5	0.64	<2	0.28	<2	0.98
Pentraxin 3/TSG-14	<2	0.43	2-5	0.21	2-5	0.40	<2	0.58
PF4 (Cxcl4)	>10	0.20	5-10	0.45	<2	0.48	<2	0.69
RAGE	>10	0.03*	5-10	0.33	<2	0.34	2-5	0.54
RANTES	2-5	0.05*	<2	0.39	<2	0.03*	<2	0.98
RBP-4	2-5	0.15	<2	0.17	<2	0.05*	<2	0.98
Relaxin-2	5-10	0.10	<2	0.13	<2	0.07	<2	0.70
Resistin	2-5	0.15	<2	0.19	<2	0.05*	<2	0.59
SDF-1α (Cxcl12)	<2	0.50	<2	0.73	<2	0.68	<2	0.62
Serpin E1/PAI-1	<2	0.35	<2	0.98	<2	0.79	<2	0.12
SHBG (ABP)	2-5	0.15	2-5	0.59	<2	0.72	<2	0.70
ST2 (IL-1 R4)	5-10	0.01*	5-10	0.43	<2	0.87	5-10	0.38
TARC (CCL17)	5-10	0.24	2-5	0.48	<2	0.44	2-5	0.51
TFF3	>10	0.31	5-10	0.38	<2	0.51	2-5	0.60
TfR (CD71)	>10	0.28	>10	0.14	<2	0.82	2-5	0.55
TGF-α	5-10	0.02*	<2	0.84	<2	0.01*	2-5	0.64
Thrombospondin-1	5-10	0.06	5-10	0.12	<2	0.54	<2	0.75
TNF-α	2-5	0.21	<2	0.21	<2	0.11	<2	0.77
uPAR	5-10	0.19	<2	0.15	<2	0.15	<2	0.71
VEGF	>10	0.06	5-10	0.46	<2	0.08	2-5	0.57
Vitamin D BP	5-10	0.18	2-5	0.34	<2	0.52	2-5	0.50
CD31 (PECAM-1)	>10	0.01*	2-5	0.19	<2	0.01*	<2	0.38
TIM-3	>10	0.01*	2-5	0.19	<2	0.02*	<2	0.83
VCAM-1/CD106	2-5	0.03*	2-5	0.11	<2	0.51	<2	0.79

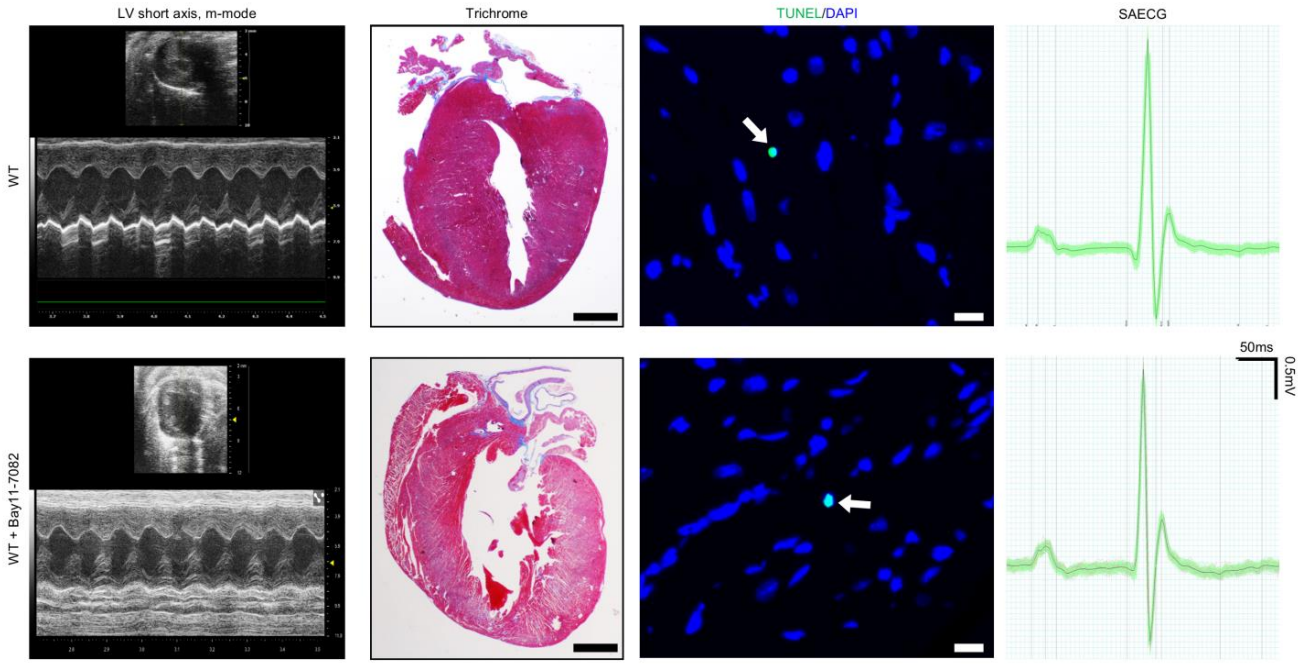
Fold Change
<2
2-5
5-10
>10
>20

Supplemental Table 3. Data are presented as fold-change; P values are shown for untreated *PKP2* hiPSC-CM supernatant vs. untreated control hiPSC-CM supernatant (columns 1 and 2); Bay 11-7082-treated *PKP2* hiPSC-CM supernatant vs. untreated control hiPSC-CM supernatant (columns 3 and 4); Bay 11-7082=treated *PKP2* hiPSC-CM supernatant vs. untreated *PKP2* hiPSC-CM supernatant (columns 5 and 6); and Bay 11-7082-treated control hiPSC-CM supernatant vs. untreated control hiPSC-CM supernatant (columns 7 and 8). P values were determined y by one-way ANOVA with Tukey's multiple comparisons test; * $P \leq 0.05$ for comparisons between cohorts within columns (e.g., *PKP2* vs. control); n=3 cell cultures for each cohort.

Supplemental Figures

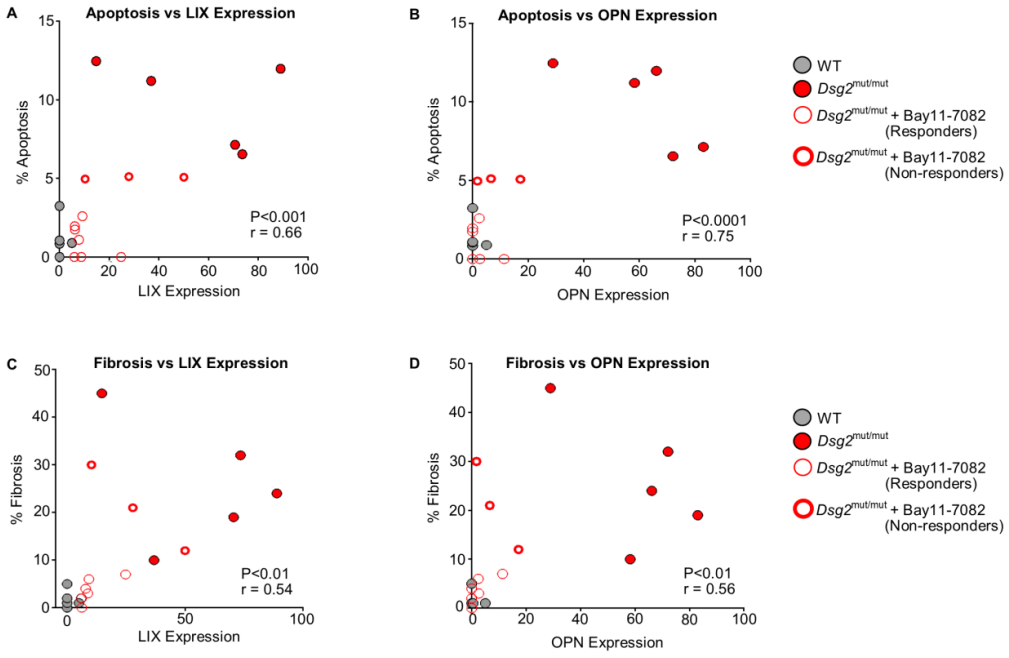


Supplemental Figure 1. Effects of Bay 11-7082 on control neonatal rat ventricular myocytes (NRVM) in vitro. Representative confocal immunofluorescence images from control (non-transfected) NRVMs in the absence or presence of Bay 11-7082 show the distribution of phospho-RelA/p65 (pRelA/p65), N-cadherin, plakoglobin, Cx43, GSK3β, and TUNEL + nuclei. Arrows show localization of immunoreactive signal at the cell surface. Arrow heads show TUNEL + nuclei. Scale bar = 50μM.



Supplemental Figure 2. Effects of Bay 11-7082 on wildtype (WT) mice in vivo.

Representative left ventricular (LV) short-axis m-mode echocardiograms, long-axis sections of hearts stained with Masson trichrome (scale bar = 1mm), TUNEL-labeled myocardium (scale bar = 10μm), and signal-averaged electrocardiograms (SAECGs) from vehicle-treated WT (n=10) mice and WT mice treated with Bay 11-7082 (n=5).



E Pearson's Correlation Matrix (p-values)

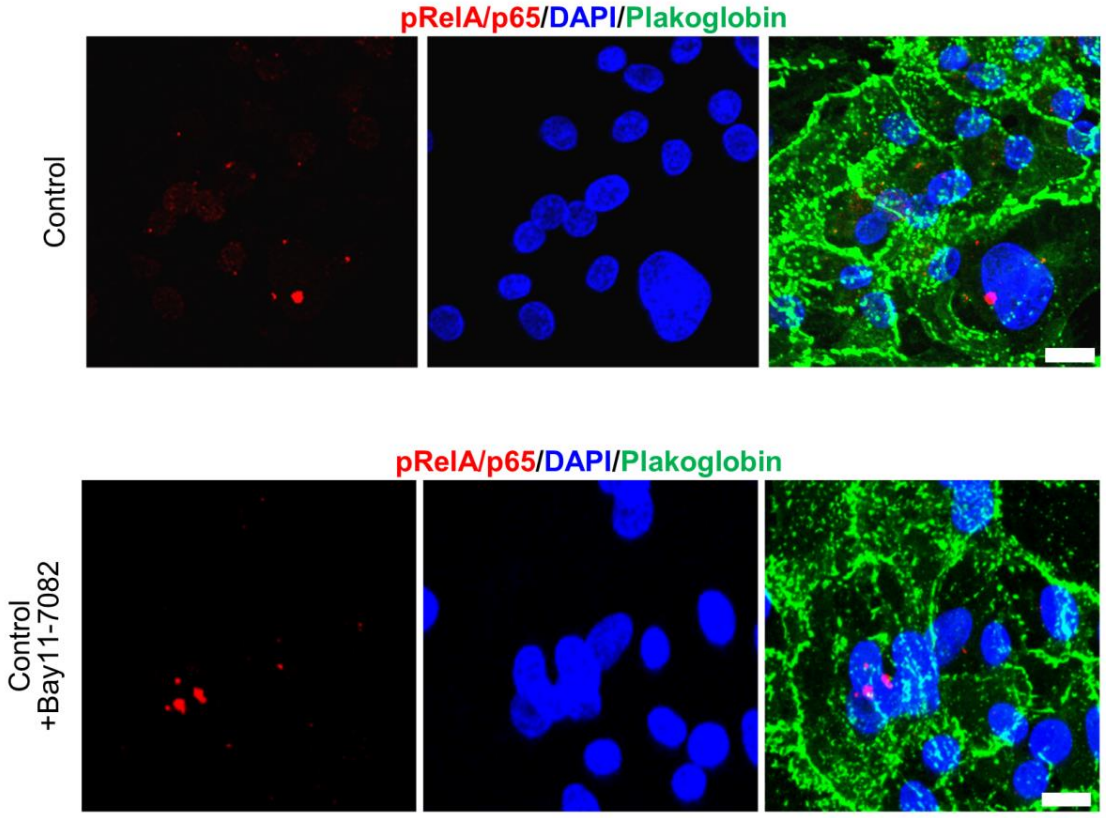
Cytokine	LIX	OPN	CCL21	CFD	DPP-IV	GAS6	IFNγ	IL-1Ra	IL-27
LIX		7.3e-08	0.046	0.019	0.002	0.059	0.010	0.018	0.005
OPN	7.3e-08		0.026	0.027	0.007	0.029	0.041	0.009	0.018
CCL-21	0.046	0.026		0.003	0.003	0.287	0.170	3.7e-4	0.030
CFD	0.019	0.027	0.003		1.4e-06	0.017	6.0e-05	0.015	2.8e-07
DPP-IV	0.002	0.007	0.003	1.4e-06		0.006	8.1e-05	0.002	9.1e-07
GAS6	0.059	0.029	0.287	0.017	0.006		0.034	0.098	0.010
IFNγ	0.010	0.041	0.170	6.0e-05	8.1e-05	0.034		0.049	2.7e-06
IL-1Ra	0.018	0.009	3.7e-4	0.015	0.002	0.098	0.049		0.028
IL-27	0.005	0.018	0.030	2.8e-07	9.1e-07	0.010	2.7e-06	0.028	

F Pearson's Correlation Matrix (r-values)

Cytokine	LIX	OPN	CCL21	CFD	DPP-IV	GAS6	IFNγ	IL-1Ra	IL-27
LIX		0.899	0.452	0.519	0.658	0.429	0.560	0.523	0.604
OPN	0.899		0.498	0.493	0.580	0.489	0.461	0.566	0.522
CCL-21	0.452	0.498		0.635	0.632	0.251	0.319	0.717	0.484
CFD	0.519	0.493	0.635		0.857	0.527	0.775	0.537	0.882
DPP-IV	0.658	0.580	0.632	0.857		0.596	0.766	0.638	0.864
GAS6	0.429	0.489	0.251	0.527	0.596		0.476	0.380	0.559
IFNγ	0.560	0.461	0.319	0.775	0.766	0.476		0.445	0.846
IL-1Ra	0.523	0.566	0.717	0.537	0.638	0.380	0.445		0.492
IL-27	0.604	0.522	0.484	0.882	0.864	0.559	0.846	0.492	

Supplemental Figure 3. Myocardial LIX and OPN correlation analyses with myocardial injury and cytokine expression. A, B. Pearson's correlation analyses of LIX (panel A) and OPN (panel B) expression level in each mouse vs. percent (%) apoptosis score. **C, D.** Pearson's

correlation analyses for LIX (panel C) and OPN (panel D) expression level in each mouse vs. percent (%) fibrotic score. A strong positive correlation was seen between expression levels of LIX and OPN and myocardial apoptosis and fibrosis independent of genotype or treatment protocol. **E, F**. Matrix of Pearson's p-values (panel E) and Pearson's correlation coefficients (panel F) showing that OPN and LIX expression levels correlated strongly with each other and with levels of CCL21, complement factor D (CFD), DPP-IV, GAS6, IFNγ, IL-1Ra, and IL-27. Quantitative data (mean ± SEM) in panels A-F are from n=5 for vehicle-treated WT mice, n=5 for vehicle-treated *Dsg2*$^{mut/mut}$ mice, and n=10 for Bay11-7082 treated *Dsg2*$^{mut/mut}$ mice. Correlation coefficients (r) and p-values were computed using two-tailed Pearson's correlation analysis.



Supplemental Figure 4. Effects of Bay 11-7082 on phospho-RelA/p65 signal distribution in control hiPSC-CMs in vitro. Representative confocal immunofluorescence images from cultures of cardiac myocytes derived from a control hiPSC cell line in the absence or presence of Bay 11-7082. Diffuse nuclear signal for pRelA/p65 is not seen. Bay 11-7082 had no effect on the normal cell surface distribution of the desmosomal protein plakoglobin. Scale bar = 20μm.

References

1. Chelko SP, Asimaki A, Andersen P, Bedja D, Amat-Alarcon N, DeMazumder D, Jasti R, MacRae CA, Leber R, Kleber AG, Saffitz JE, Judge DP. Central role for GSK3β in the pathogenesis of arrhythmogenic cardiomyopathy. *JCI Insight*. 2016; 1(5). pii: e85923.

2. Asimaki A, Kapoor S, Plovie E, Karin Arndt A, Adams E, Liu Z, James CA, Judge DP, Calkins H, Churko J, Wu JC, MacRae CA, Kléber AG, Saffitz JE. Identification of a new modulator of the intercalated disc in a zebrafish model of arrhythmogenic cardiomyopathy. *Sci Transl Med*. 2014; 6:240ra74.

3. Asimaki A, Tandri H, Duffy ER, Winterfield JR, Mackey-Bojack S, Picken MM, Cooper LT, Wilber DJ, Marcus FI, Basso C, Thiene G, Tsatsopoulou A, Protonotarios N, Stevenson WG, McKenna WJ, Gautam S, Remick DG, Calkins, Saffitz JE. Altered desmosomal proteins in granulomatous myocarditis and potential pathogenic links to arrhythmogenic right ventricular cardiomyopathy. *Circ Arrhyth Electrophysiol*. 2011; 4:743-52.