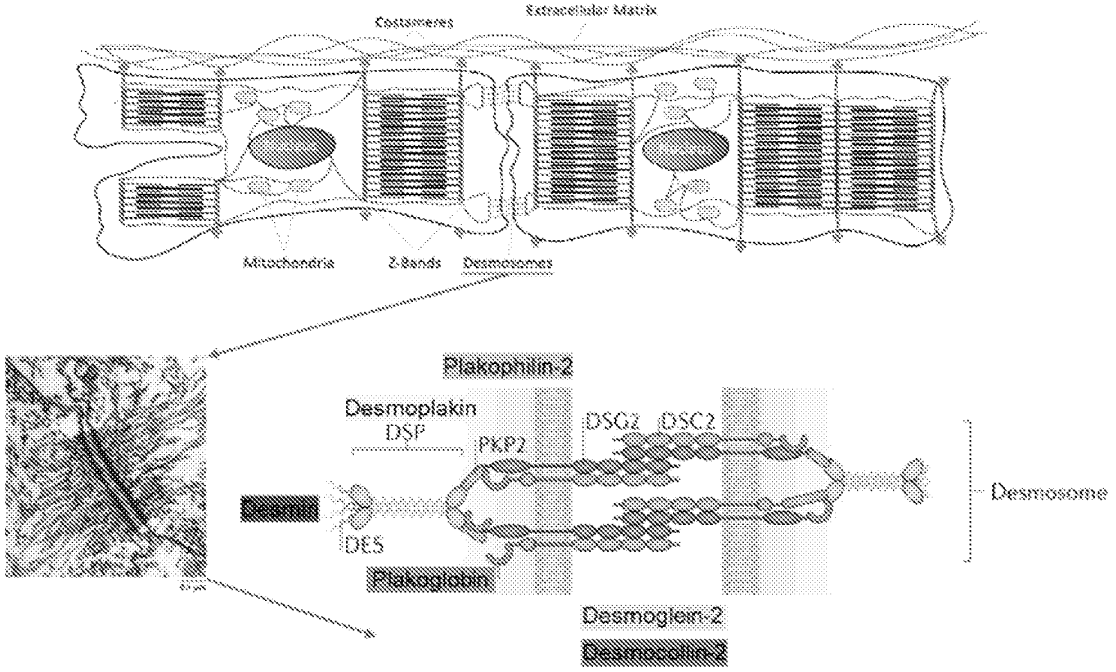


FIG. 1

Dilation and systolic/diastolic defects

- Mainly affect the right ventricle but can be biventricular
  - Mouse cKO model showing a biventricular dilation and make it easier to study AAV transgene data
- Impacted sarcomere-mediated tension generation, reduced cell-cell communication due to degradation of desmosome quality
  - Mis-regulation of sarcomere genes impacted on sarcomere quality
  - Mis-regulation of Ca^{2+} and Na^{+} channel genes impacted on electrical coupling
- Cell death
  - Myocardial atrophy
- Cardiomyocyte de-differentiation or infiltration due to death of cardiomyocytes:
  - Mis-regulation of lipogenic and fibrotic genes

Arrhythmias, conduction delays, electrical instabilities

- Ca^{2+} mishandling and other ion channel defects
  - Mouse model PKP2 cKO has an arrhythmia phenotype due to Ca^{2+} mishandling
- Reduction of GJ protein Connexin 43
- Fibrofatty tissue leading to conduction defects
  - Expression of lipogenic or fibrotic genes in iPSCM

Fibrofatty tissue replacement

- Lipogenesis in long-term cultured patient-derived iPSCM
- Mouse PKP2 cKO only has fibrosis phenotype

FIG. 2

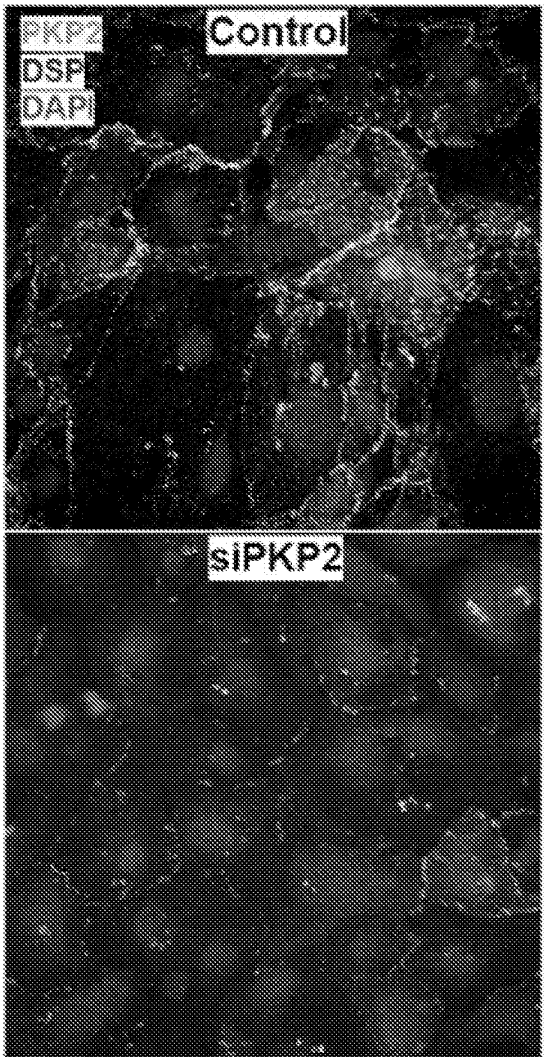


FIG. 3A

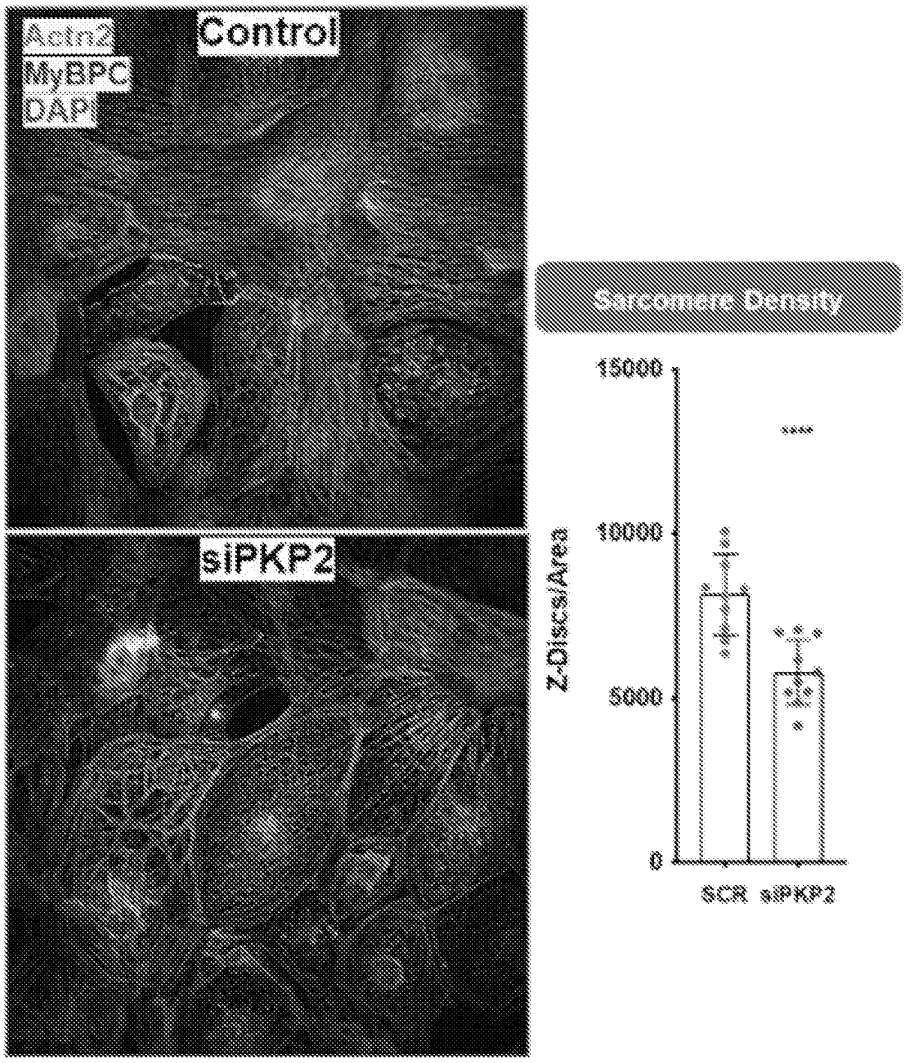

Actn2
MyBPC
DAPI
Control
siPKP2
Sarcomere Density
Z-Discs/Area
15000
10000
5000
0
SCR
siPKP2

FIG. 3B

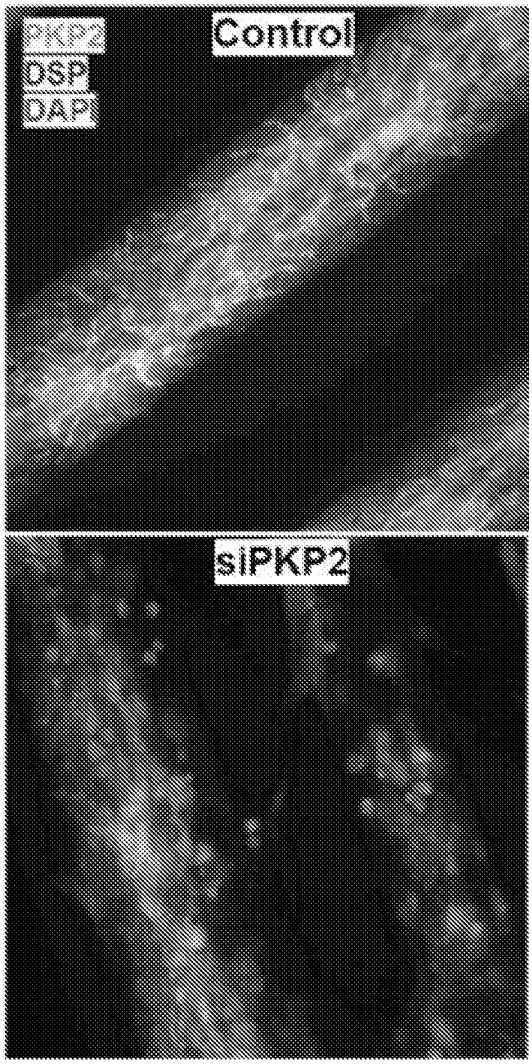


FIG. 3C

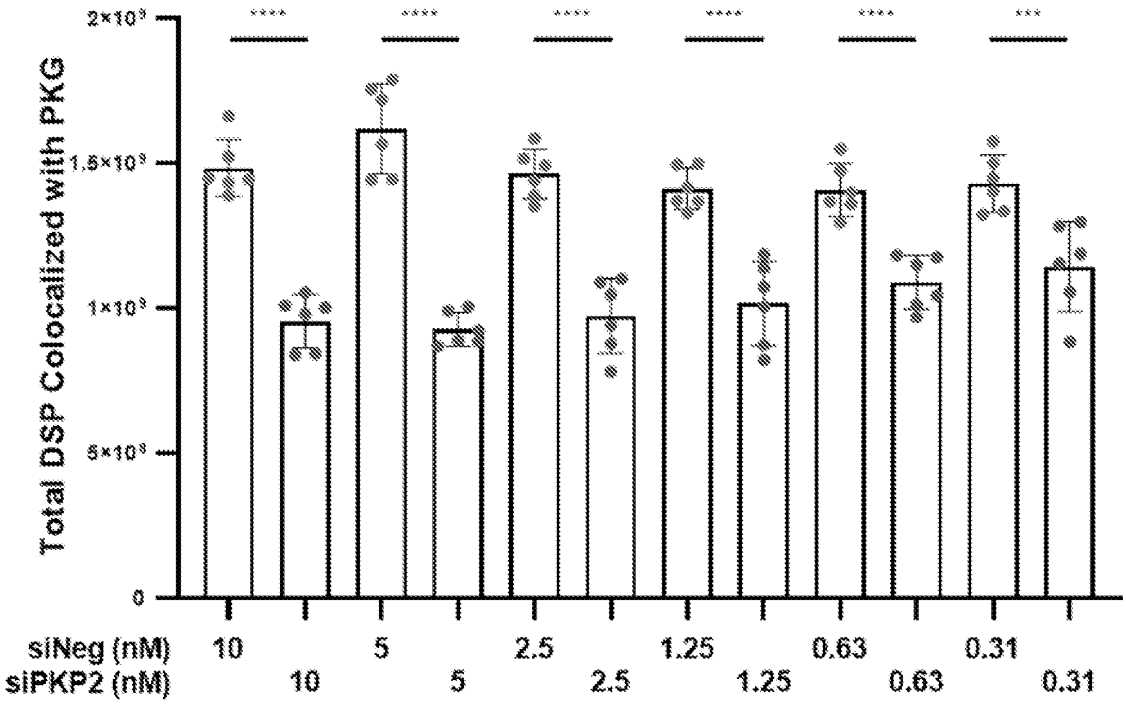

Total DSP Colocalized with PKG
2×10⁹
1.5×10⁹
1×10⁹
5×10⁸
0
****
****
****
****
****
***
siNeg (nM) 10 5 2.5 1.25 0.63 0.31
siPKP2 (nM) 10 5 2.5 1.25 0.63 0.31

FIG. 4

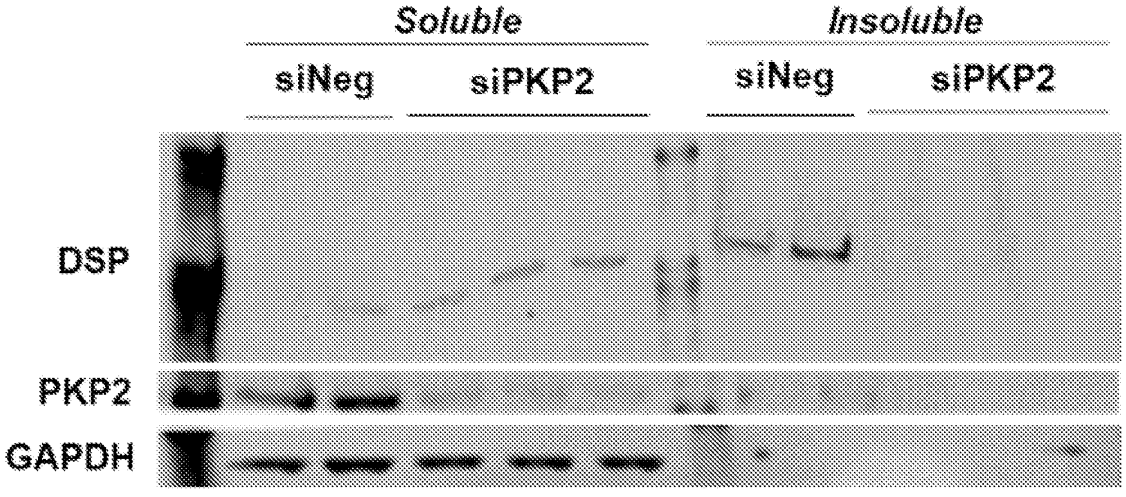

Soluble
Insoluble
siNeg
siPKP2
siNeg
siPKP2
DSP
PKP2
GAPDH

FIG. 5

AAV-pcTnT600-hPKP2α (4530 nts)
ITR
pcTnT
Kozak
PKP2α
PT2A
EGFP
pA
ITR

FIG. 6A

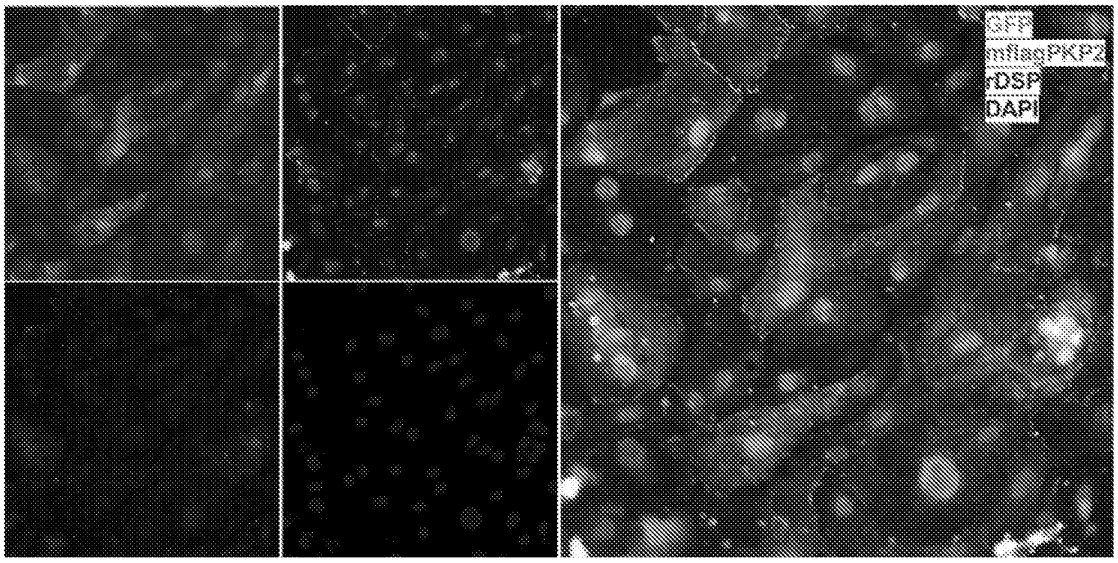

GFP
mflagPKP2
rDSP
DAPI

FIG. 6B

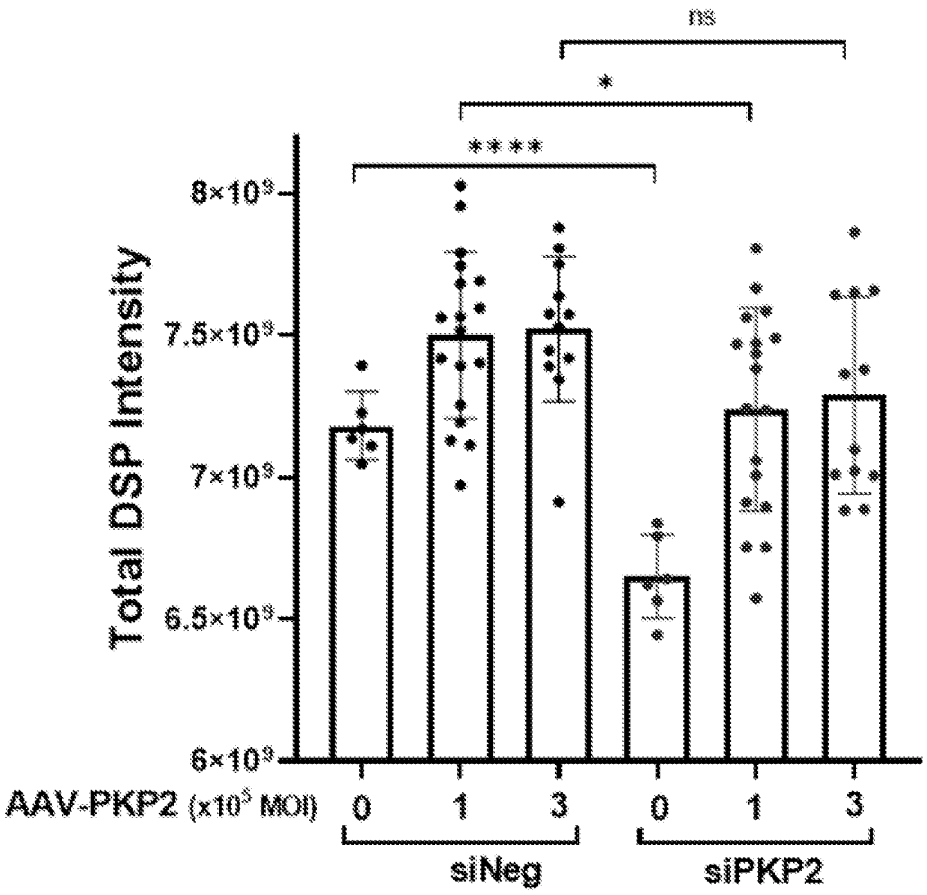

ns
*
****
Total DSP Intensity
8×10⁸
7.5×10⁸
7×10⁸
6.5×10⁸
6×10⁸
AAV-PKP2 (x10⁵ MOI)
0
1
3
0
1
3
siNeg
siPKP2

FIG. 6C

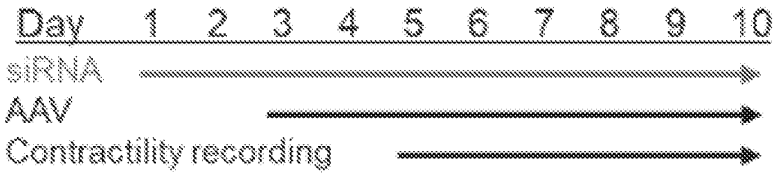

Day 1 2 3 4 5 6 7 8 9 10
siRNA
AAV
Contractility recording

FIG. 7A

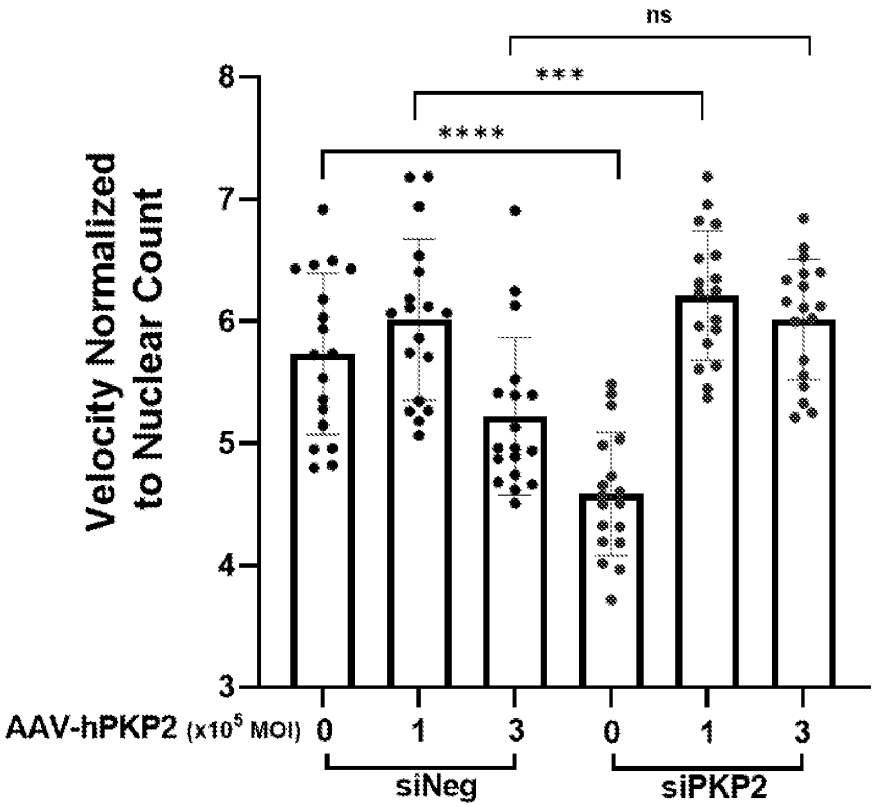


FIG. 7B

AAV Cassette Elements	Size (nt)
ITR	260
cTnT promoter	600
hPKP2α_op	2514
mPKP2α	2387
WPRE	540
bGH poly(A)	215

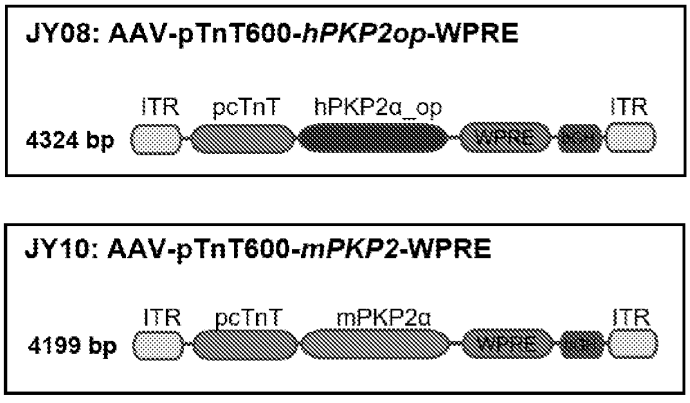


FIG. 8

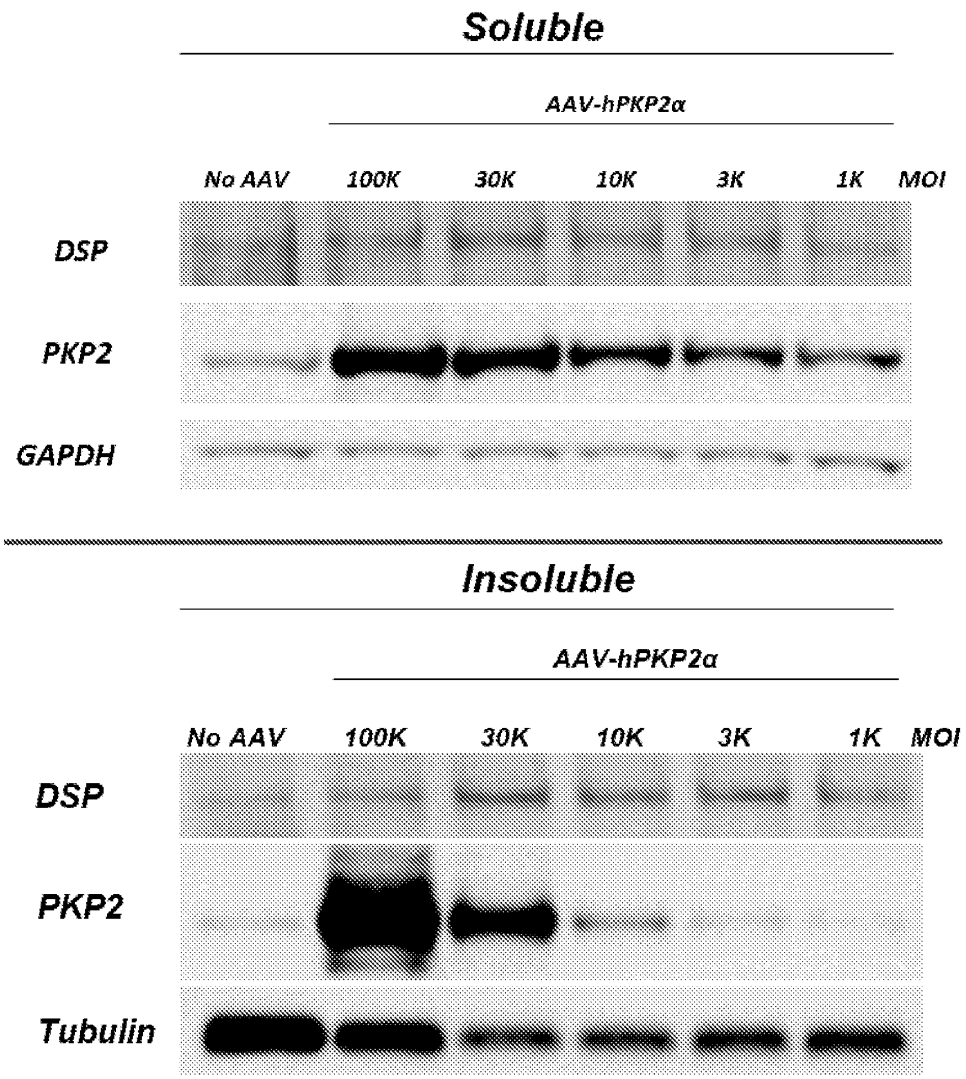

Soluble
AAV-hPKP2α
No AAV
100K
30K
10K
3K
1K
MOI
DSP
PKP2
GAPDH
Insoluble
AAV-hPKP2α
No AAV
100K
30K
10K
3K
1K
MOI
DSP
PKP2
Tubulin

FIG. 9A

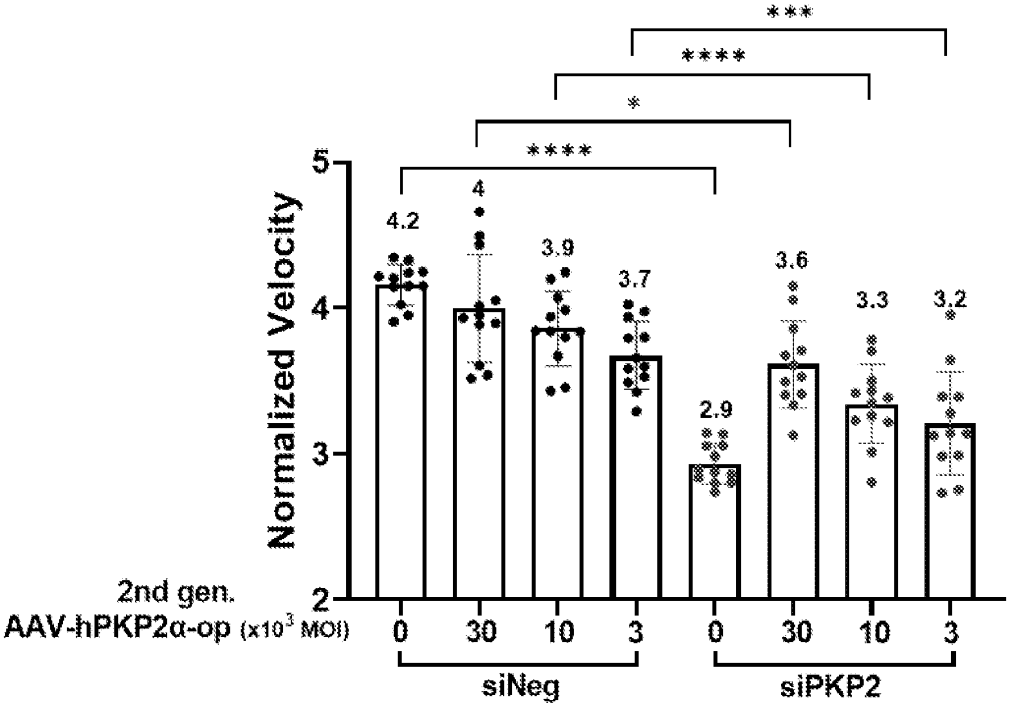

***
****
*
****
Normalized Velocity
5
4
3
2
4.2
4
3.9
3.7
2.9
3.6
3.3
3.2
2nd gen.
AAV-hPKP2α-op (x10^3 MOI)
0
30
10
3
0
30
10
3
siNeg
siPKP2

FIG. 9B

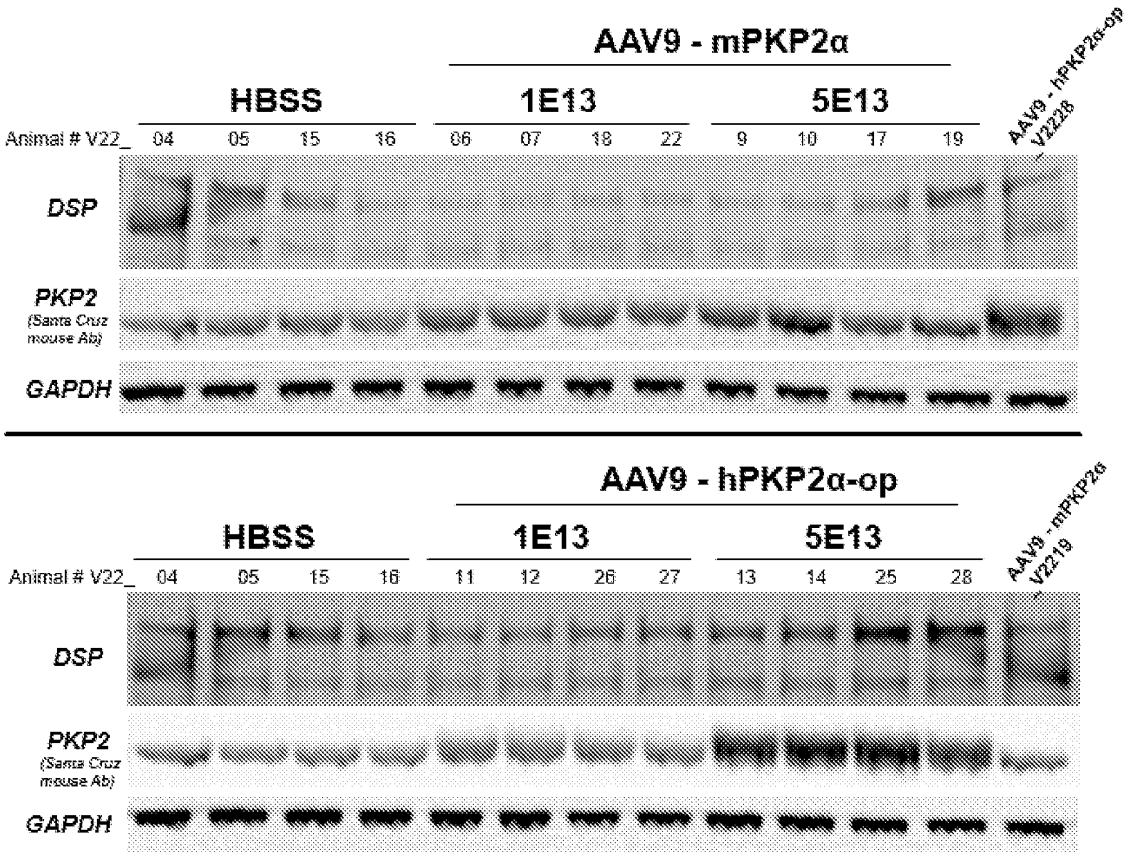


FIG. 10

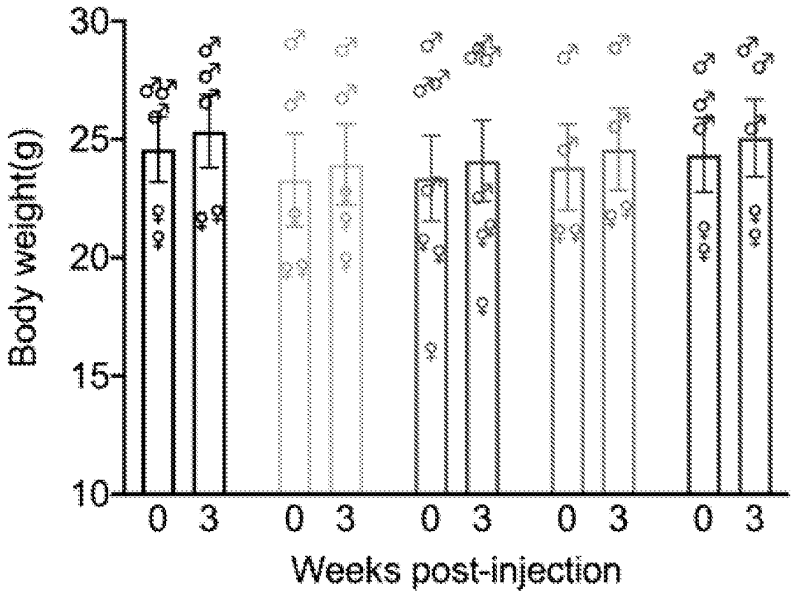


FIG. 11A

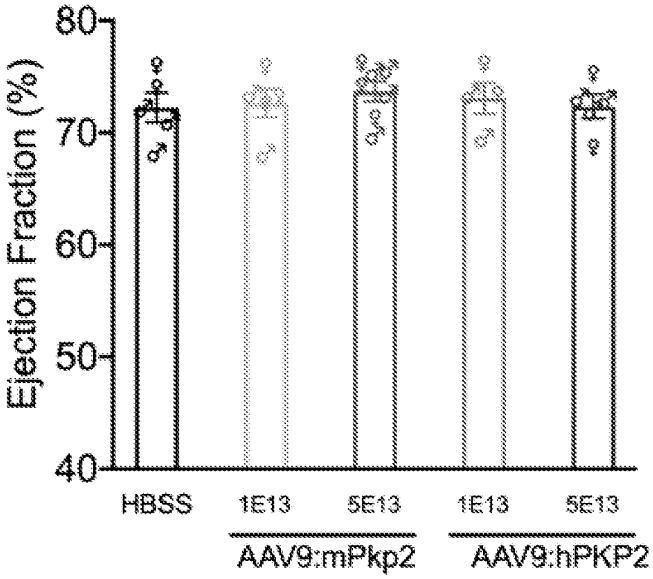


FIG. 11B

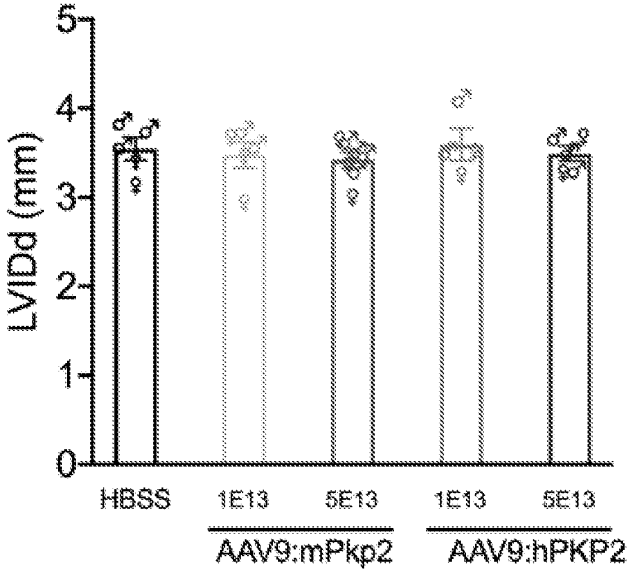


FIG. 11C

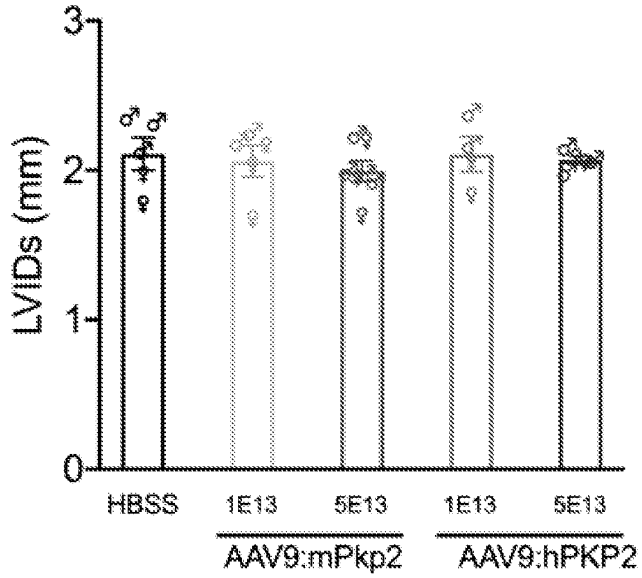


FIG. 11D

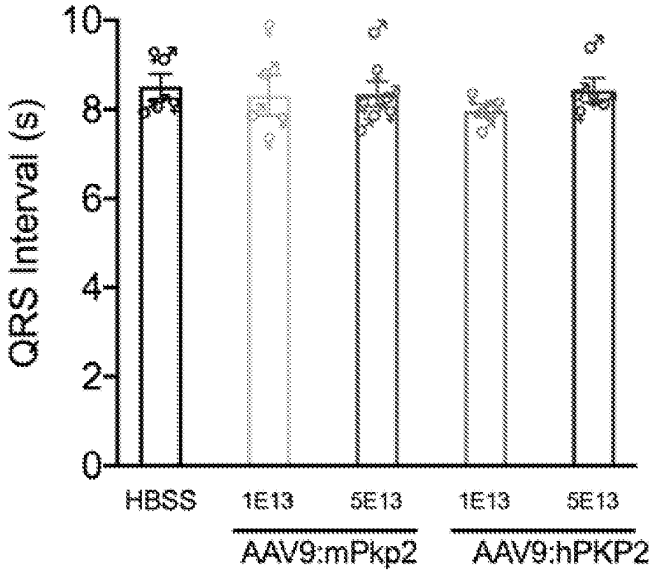


FIG. 11E

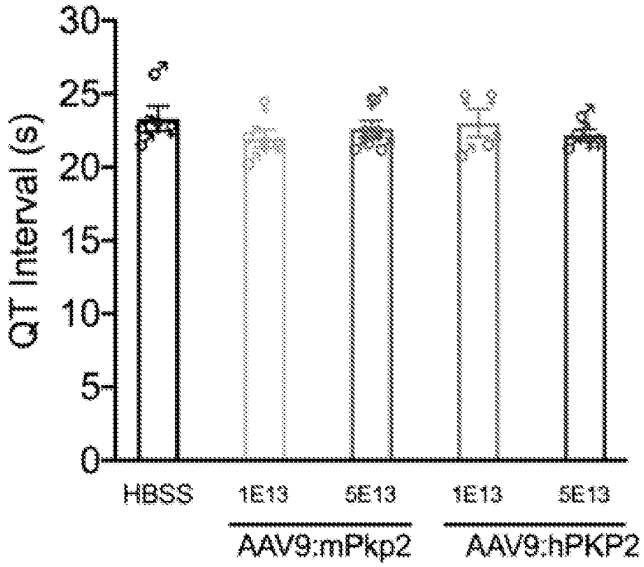


FIG. 11F

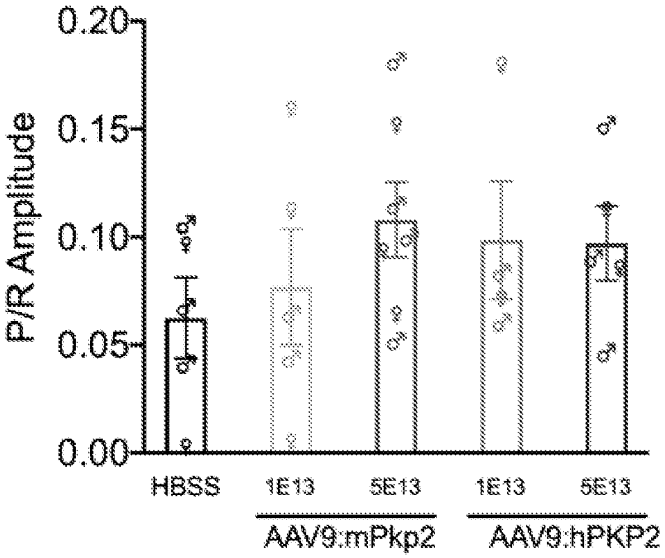


FIG. 11G

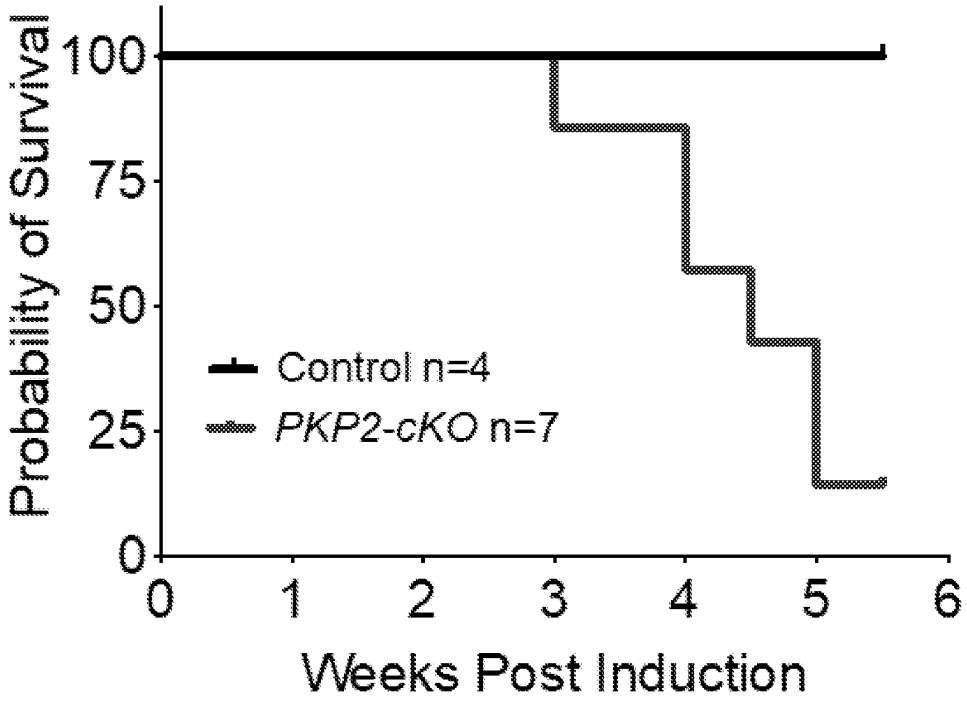


FIG. 12

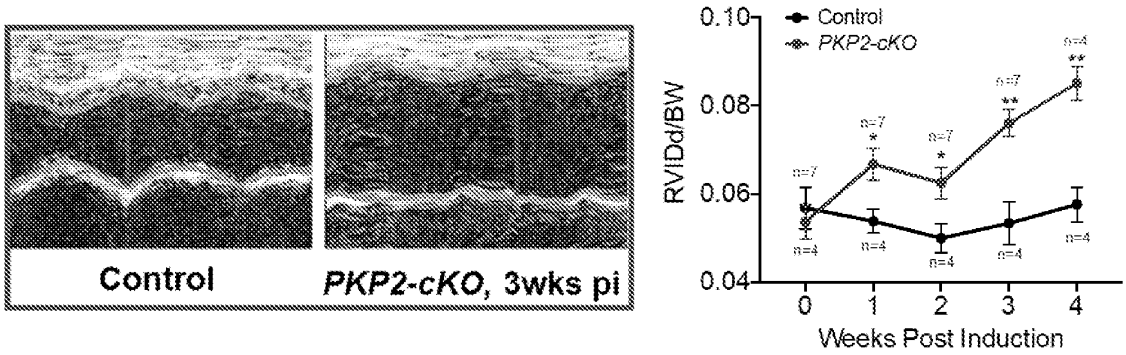


FIG. 13A

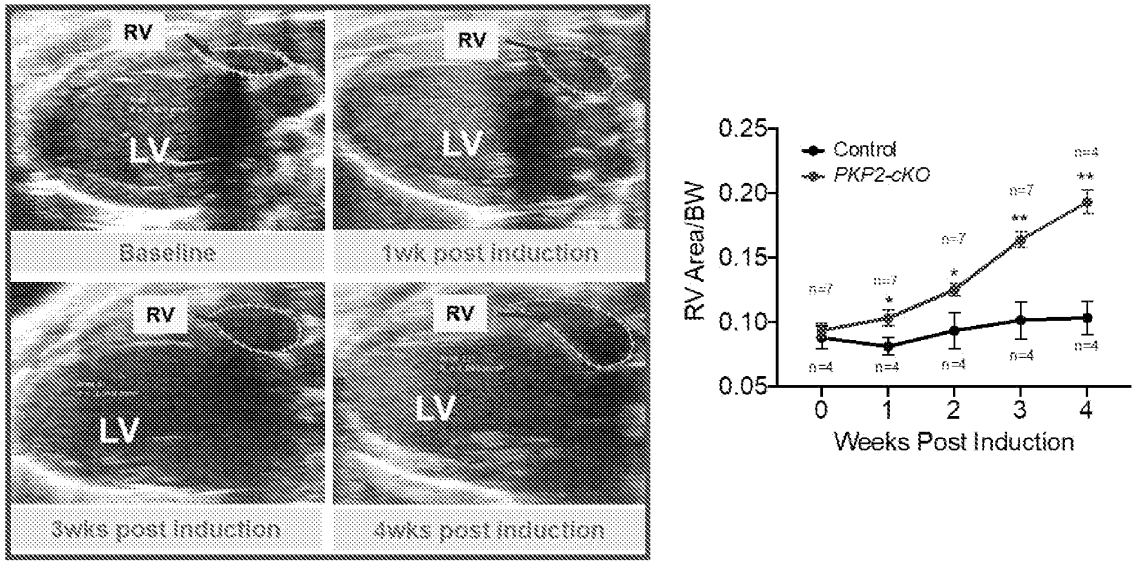


FIG. 13B

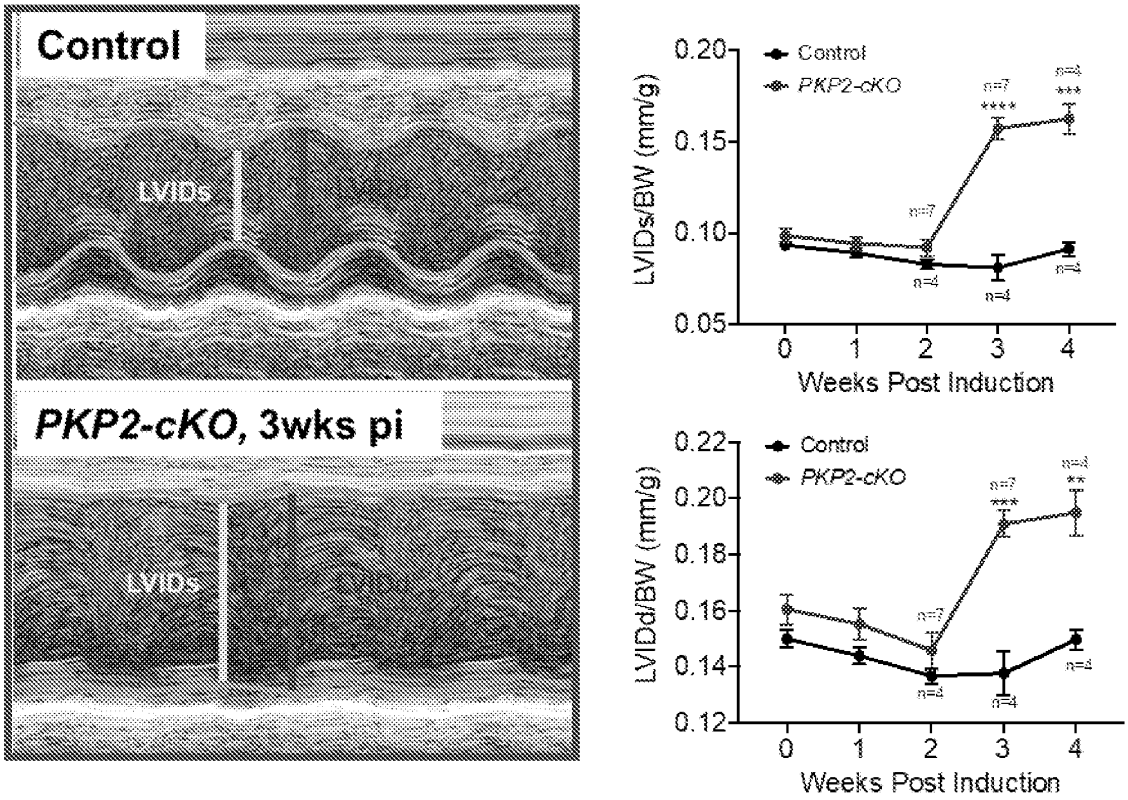


FIG. 14A

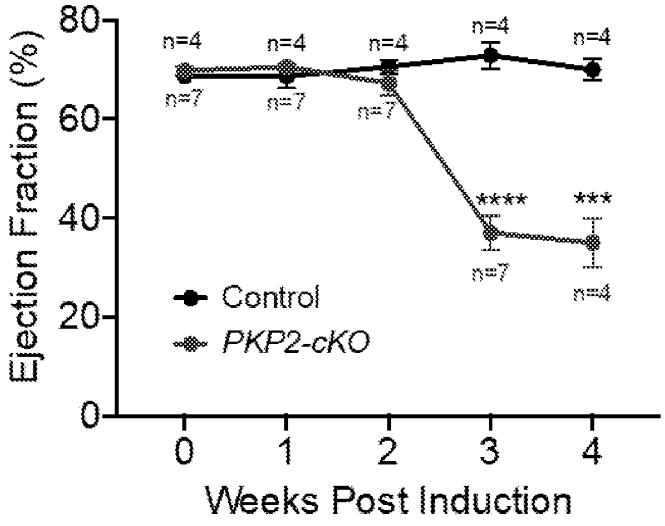

Ejection Fraction (%)
80
60
40
20
0
n=4
n=4
n=4
n=4
n=4
n=7
n=7
n=7
****
***
n=7
n=4
Control
PKP2-cKO
0
1
2
3
4
Weeks Post Induction

FIG. 14B

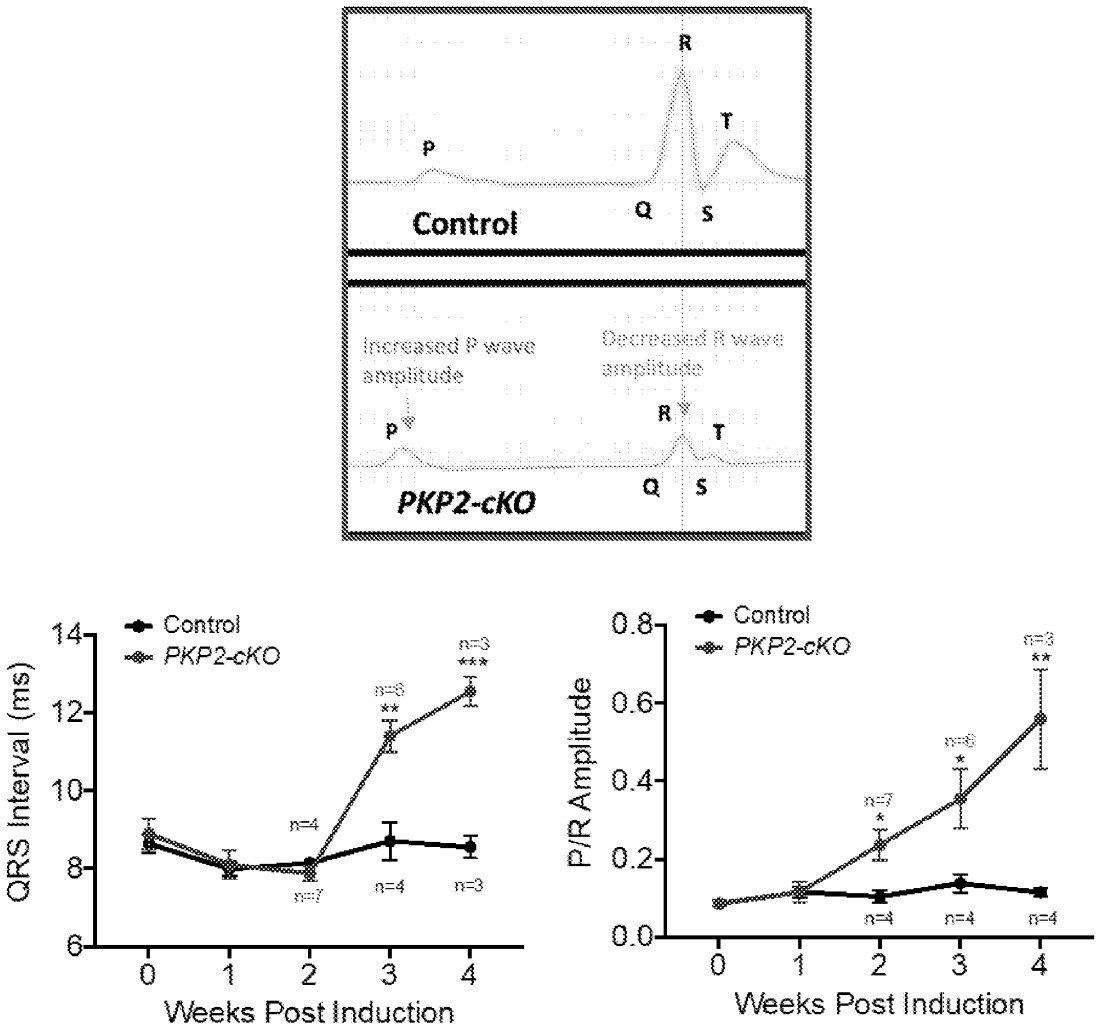
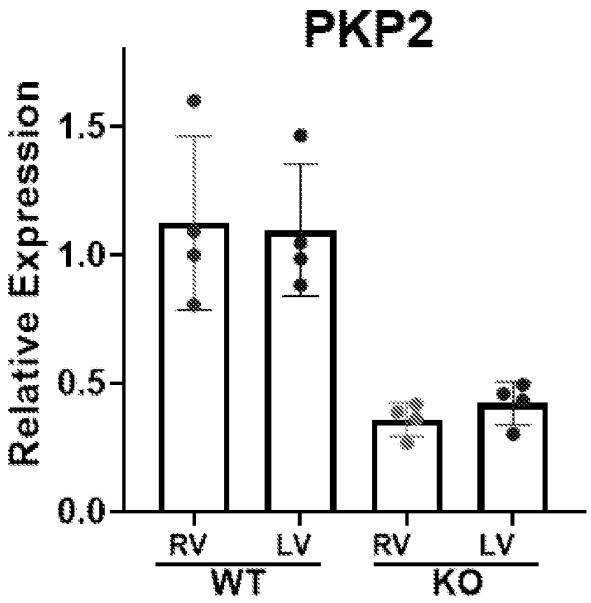

R
T
P
Control
Q
S
Increased P wave amplitude
Decreased R wave amplitude
P
R
T
PKP2-cKO
Q
S
Control
PKP2-cKO
QRS Interval (ms)
14
12
10
8
6
0
1
2
3
4
Weeks Post Induction
n=3
***
n=6
**
n=4
n=7
n=4
n=3
Control
PKP2-cKO
P/R Amplitude
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
Weeks Post Induction
n=3
**
n=6
*
n=7
*
n=4
n=4
n=4

FIG. 15


PKP2
Relative Expression
1.5
1.0
0.5
0.0
RV
LV
RV
LV
WT
KO

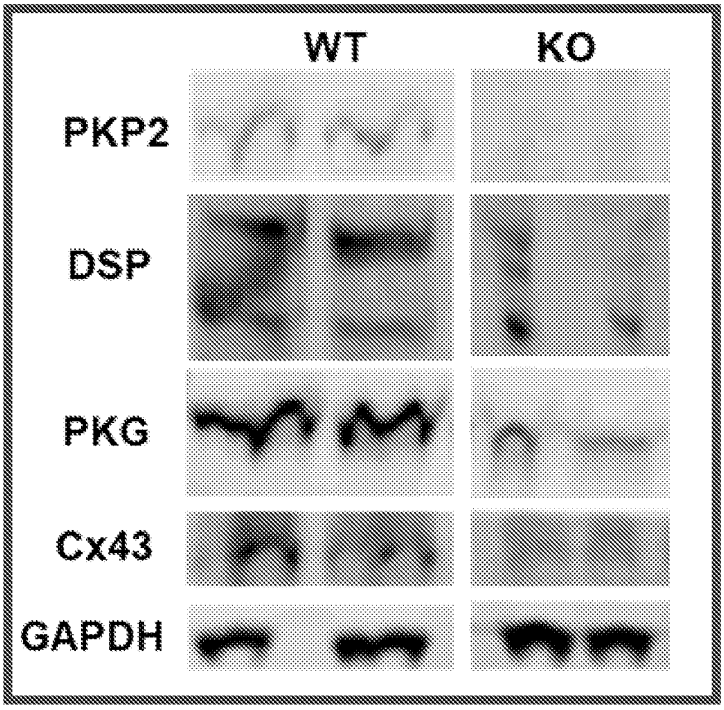

WT
KO
PKP2
DSP
PKG
Cx43
GAPDH

FIG. 16A

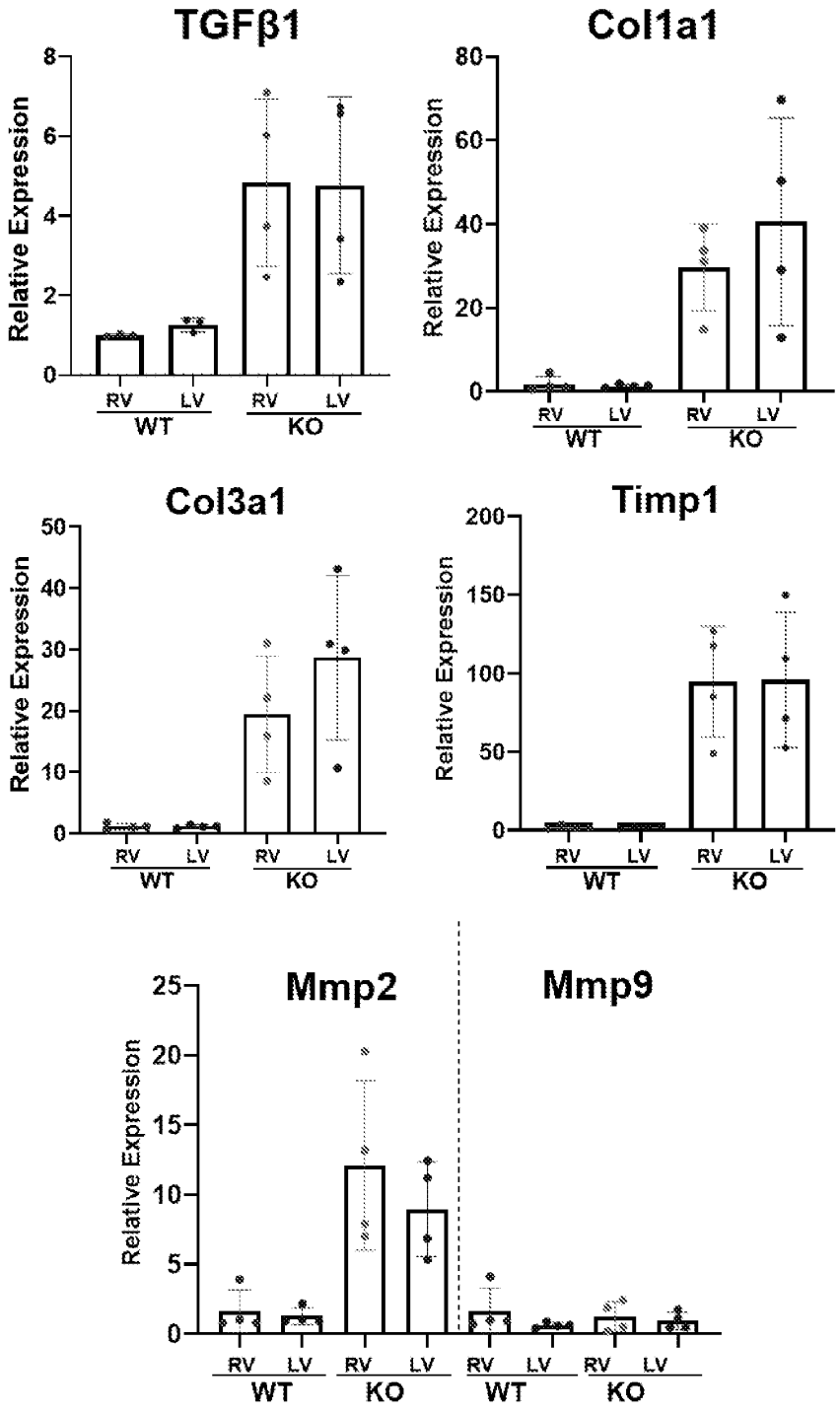

TGFβ1
Relative Expression
0
2
4
6
8
RV
LV
RV
LV
WT
KO
Col1a1
Relative Expression
0
20
40
60
80
RV
LV
RV
LV
WT
KO
Col3a1
Relative Expression
0
10
20
30
40
50
RV
LV
RV
LV
WT
KO
Timp1
Relative Expression
0
50
100
150
200
RV
LV
RV
LV
WT
KO
Mmp2
Mmp9
Relative Expression
0
5
10
15
20
25
RV
LV
RV
LV
RV
LV
RV
LV
WT
KO
WT
KO

FIG. 16B

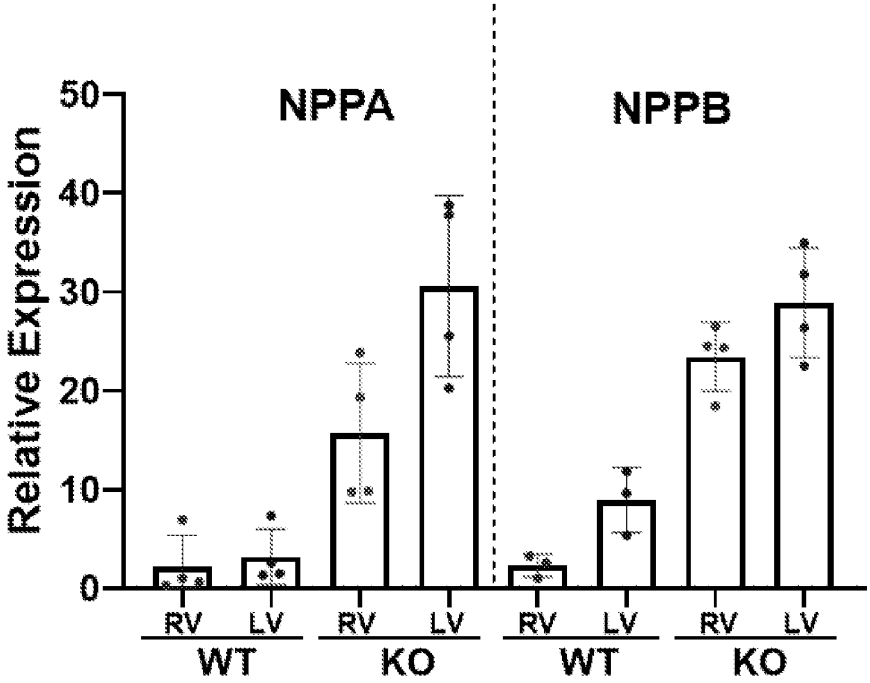
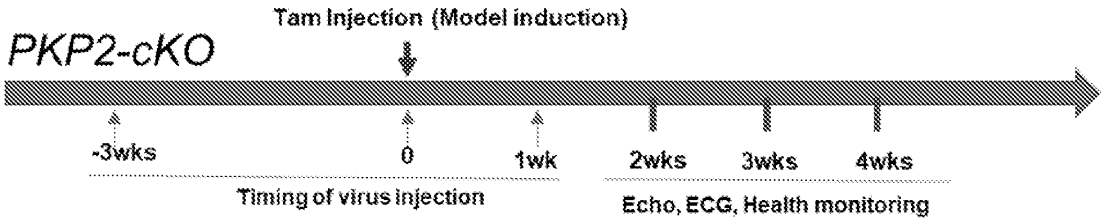


FIG. 16C



Group	Test article	Timing of intervention (relative to Tam injection)	Dose	Number
WT	HBSS	N/A	N/A	6
PKP2-cKO ARVC	HBSS	N/A	N/A	10
	AAV9: PKP2 (human)	3 weeks before	3E13	10
	AAV9: PKP2 (mouse)	3 weeks before	5E13	10
		Right after		10
		1 week after		10

FIG. 17

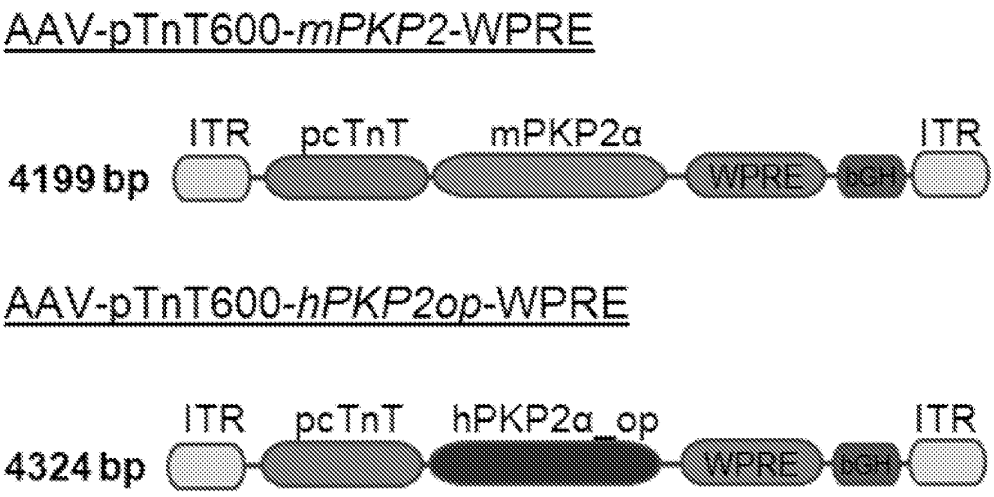


FIG. 18A

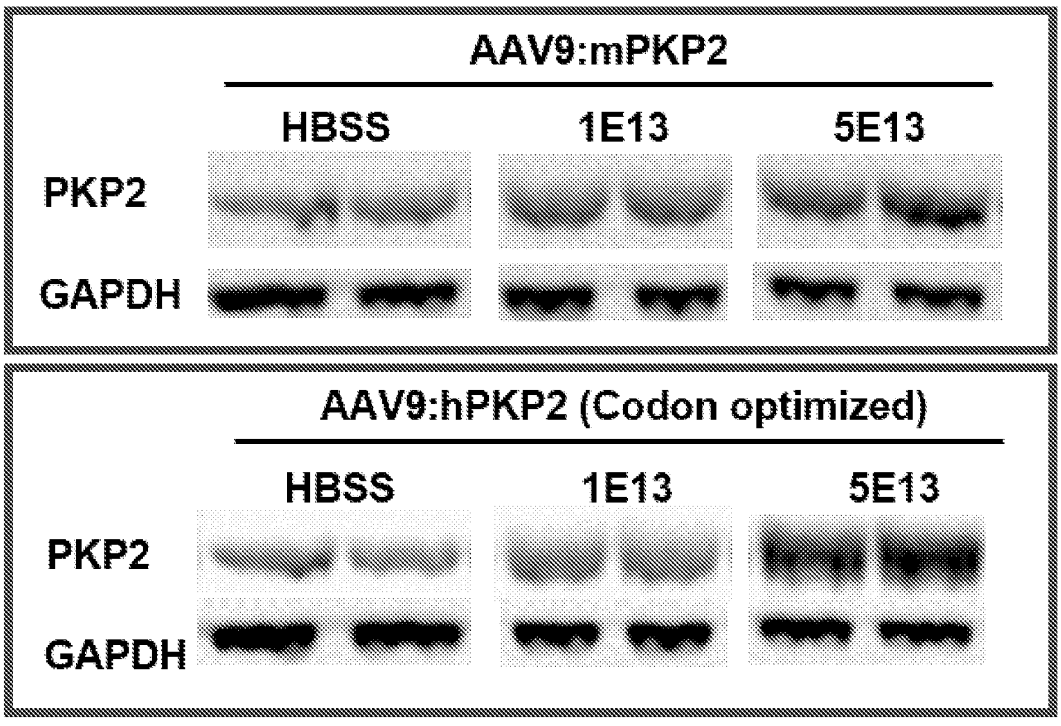


FIG. 18B

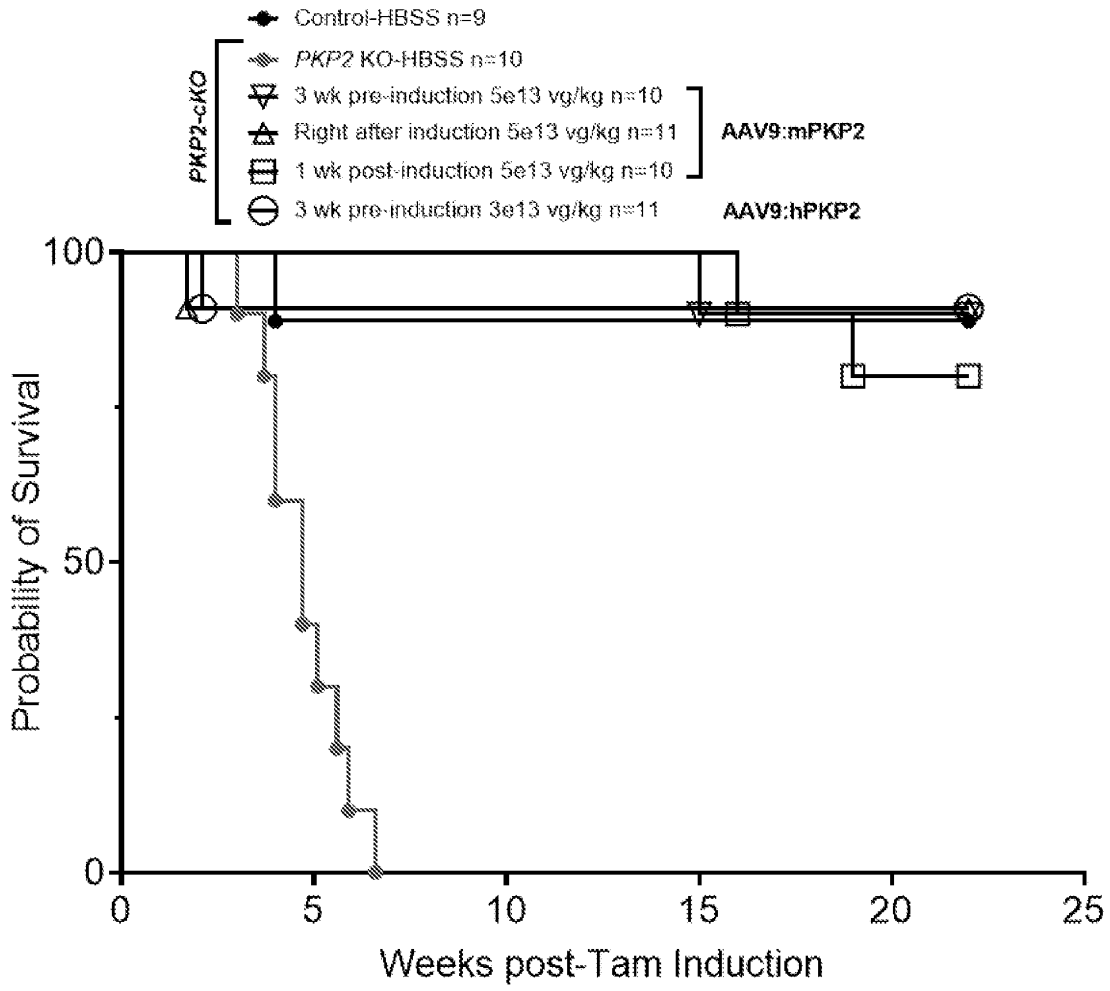

Control-HBSS n=9
PKP2 KO-HBSS n=10
3 wk pre-induction 5e13 vg/kg n=10
Right after induction 5e13 vg/kg n=11
1 wk post-induction 5e13 vg/kg n=10
3 wk pre-induction 3e13 vg/kg n=11
PKP2-cKO
AAV9:mPKP2
AAV9:hPKP2
100
50
0
Probability of Survival
0
5
10
15
20
25
Weeks post-Tam Induction

FIG. 19

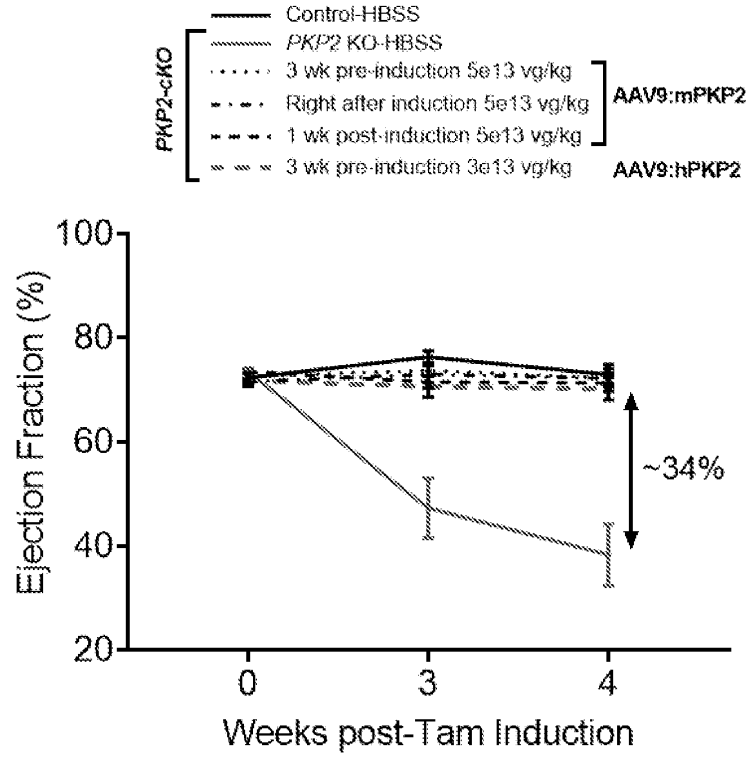


FIG. 20A

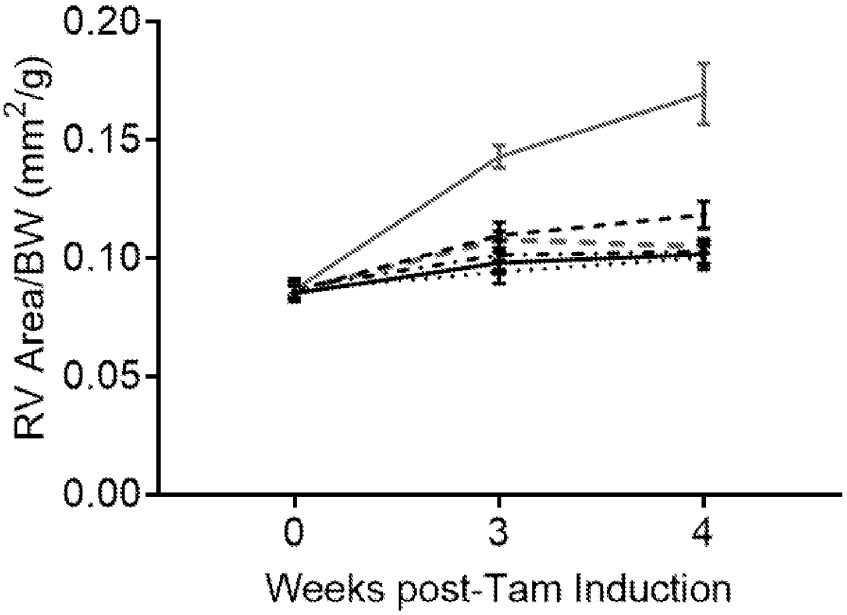


FIG. 20B

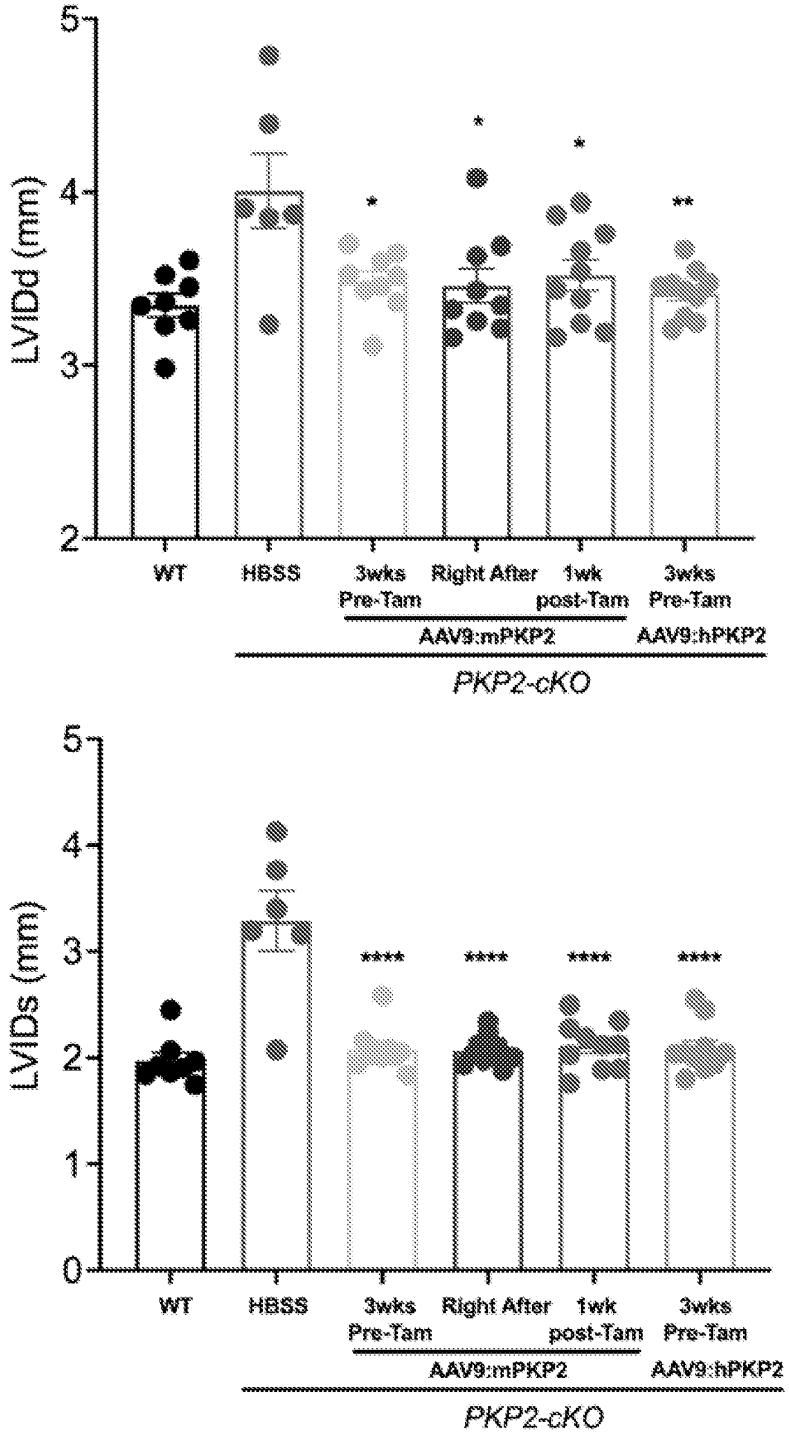

LVIDd (mm)
5
4
3
2
*
*
*
**
WT
HBSS
3wks Pre-Tam
Right After
1wk post-Tam
3wks Pre-Tam
AAV9:mPKP2
AAV9:hPKP2
PKP2-cKO
LVIDs (mm)
5
4
3
2
1
0
****
****
****
****
WT
HBSS
3wks Pre-Tam
Right After
1wk post-Tam
3wks Pre-Tam
AAV9:mPKP2
AAV9:hPKP2
PKP2-cKO

FIG. 20C

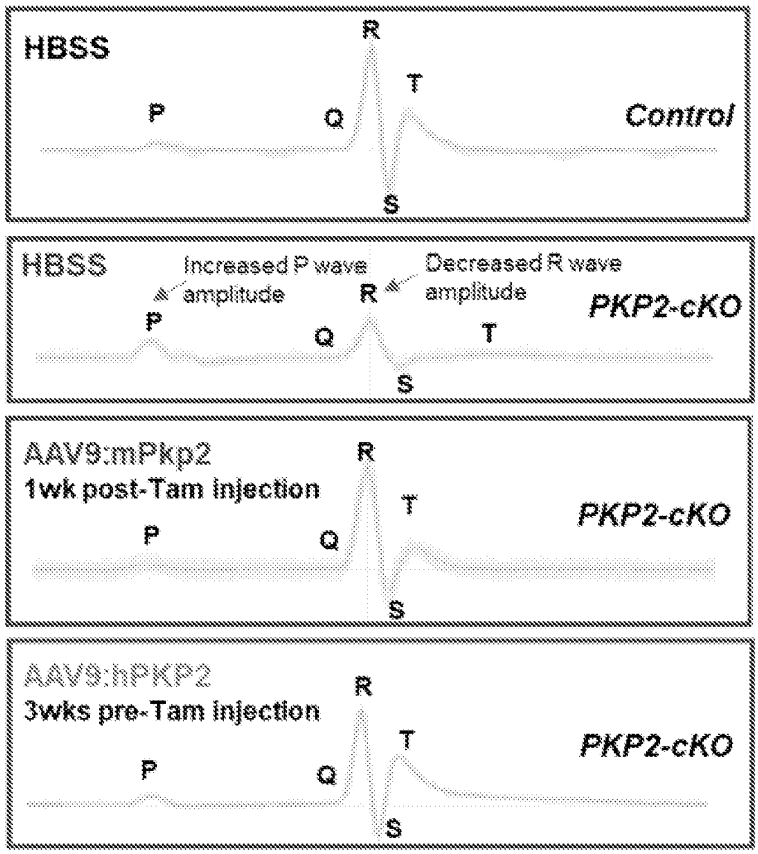

HBSS
R
T
P
Q
Control
S
HBSS
Increased P wave amplitude
Decreased R wave amplitude
R
P
PKP2-cKO
Q
T
S
AAV9:mPkp2
1wk post-Tam injection
R
T
P
PKP2-cKO
Q
S
AAV9:hPKP2
3wks pre-Tam injection
R
T
P
PKP2-cKO
Q
S

FIG. 21A

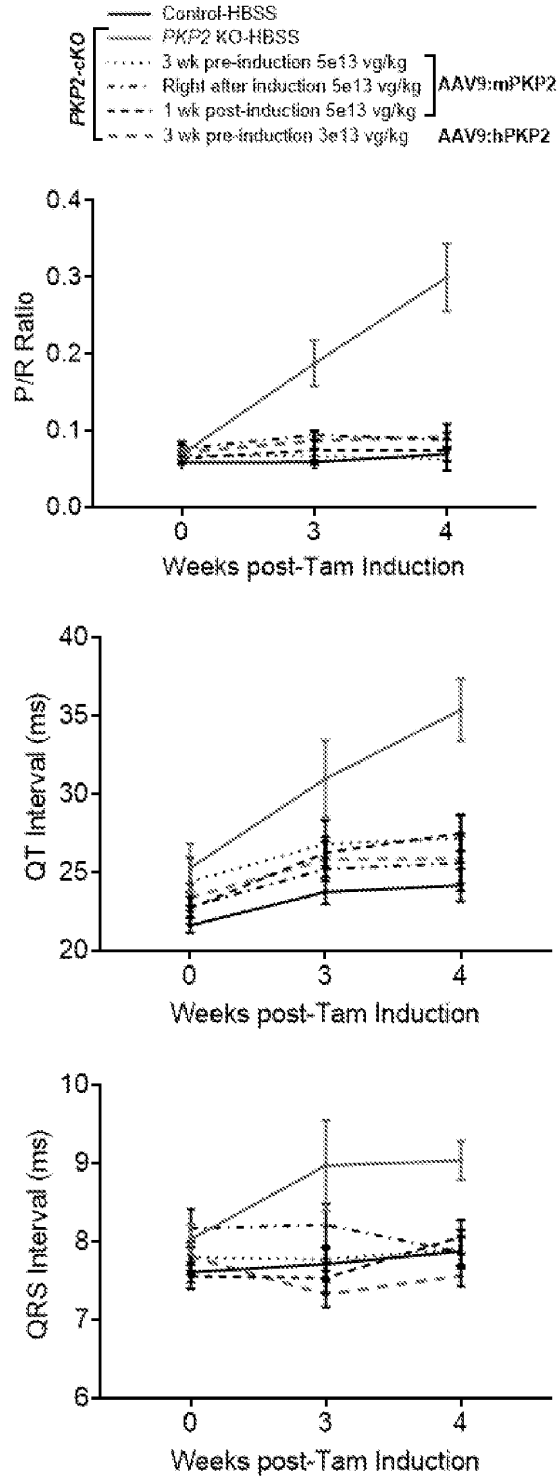

Control-HBSS
PKP2 KO-HBSS
PKP2-cKO
3 wk pre-induction 5e13 vg/kg
Right after induction 5e13 vg/kg
1 wk post-induction 5e13 vg/kg
AAV9:mPKP2
3 wk pre-induction 3e13 vg/kg
AAV9:hPKP2
P/R Ratio
0.4
0.3
0.2
0.1
0.0
0
3
4
Weeks post-Tam Induction
QT Interval (ms)
40
35
30
25
20
0
3
4
Weeks post-Tam Induction
QRS Interval (ms)
10
9
8
7
6
0
3
4
Weeks post-Tam Induction

FIG. 21B

Grade	Arrhythmias
5	S-VT/VF/Cardiac sudden death
4	NSVT
3	>100PVCs, couplets and triplets
2	>50, <100 PVCs
1	<50 PVCs, PJCs, AV Block
0	<10 PVCs

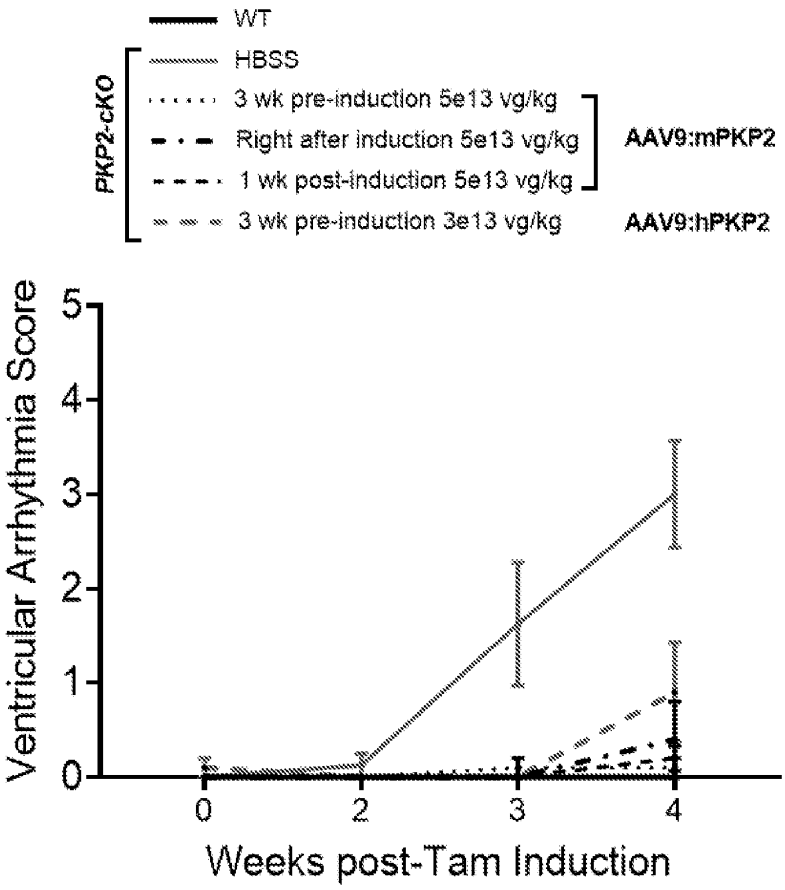


FIG. 22A

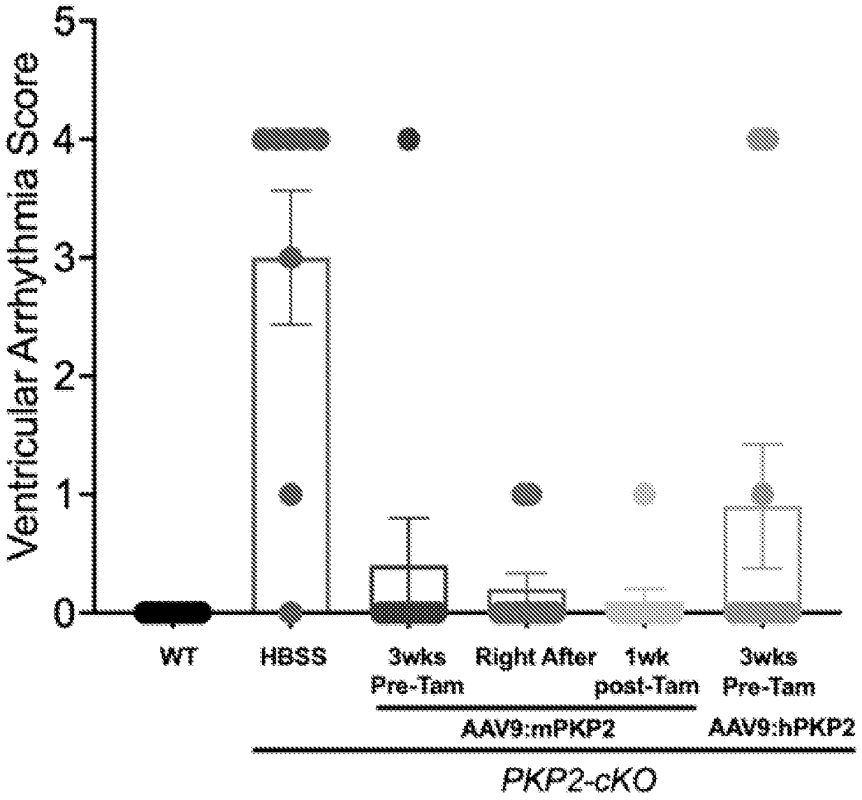
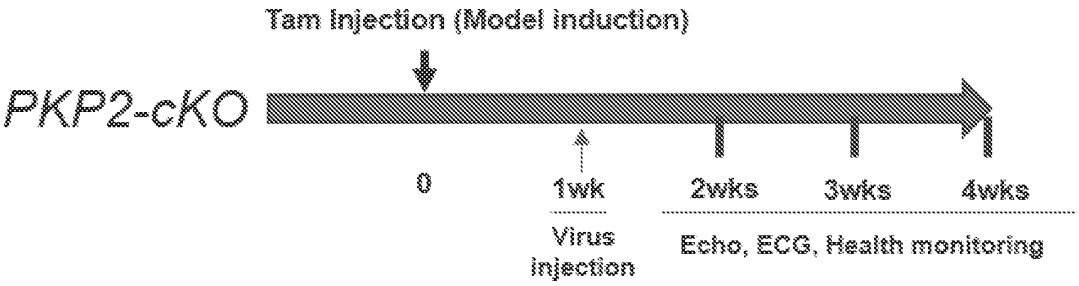


FIG. 22B



Group	Test article	Dose	Number
WT	HBSS	N/A	4
PKP2-cKO ARVC	HBSS	N/A	4
	AAV9: hPKP2 (human orthologue)	1e13	4
		3e13	3
		1e14	3

FIG. 23

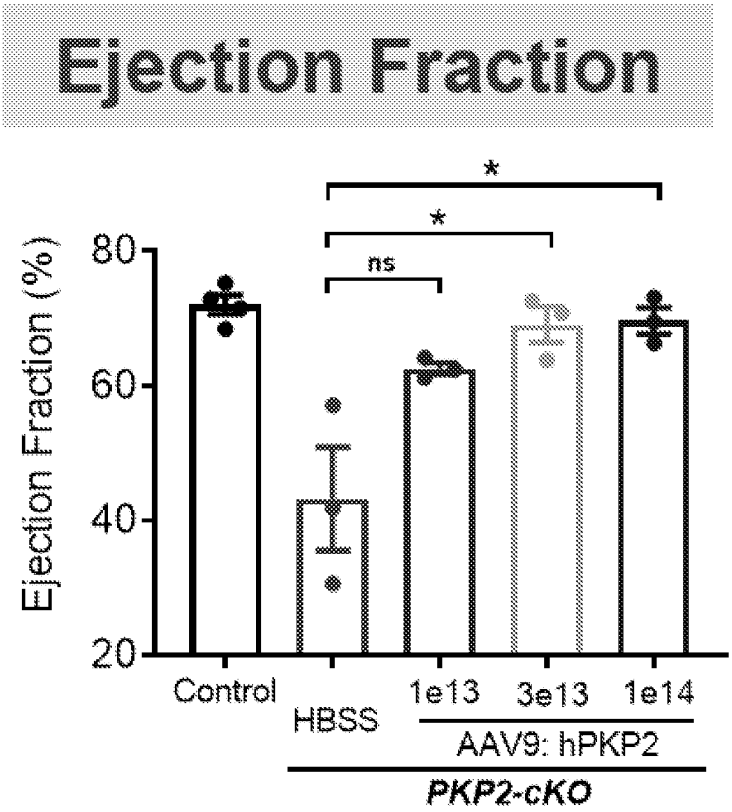

Ejection Fraction
Ejection Fraction (%)
80
60
40
20
ns
*
*
Control
HBSS
1e13
3e13
1e14
AAV9: hPKP2
PKP2-cKO

FIG. 24A

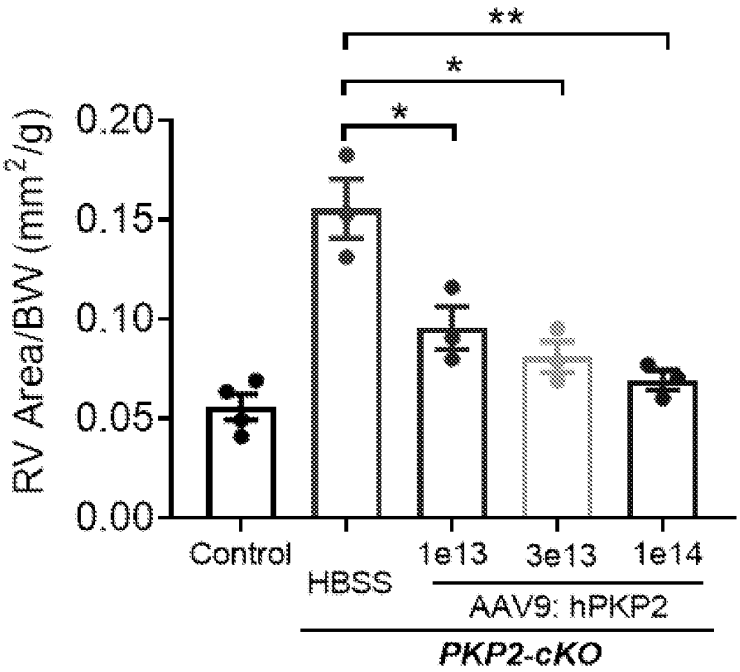

RV Size
**
*
*
RV Area/BW (mm²/g)
0.20
0.15
0.10
0.05
0.00
Control
HBSS
1e13
3e13
1e14
AAV9: hPKP2
PKP2-cKO

FIG. 24B

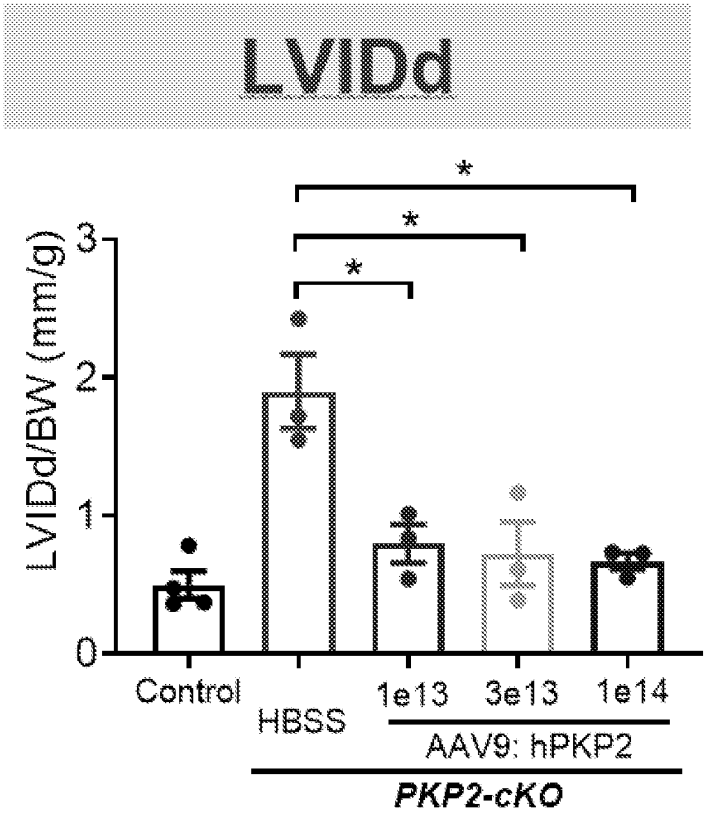

LVIDd
LVIDd/BW (mm/g)
0
1
2
3
*
*
*
Control
HBSS
1e13
3e13
1e14
AAV9: hPKP2
PKP2-cKO

FIG. 24C

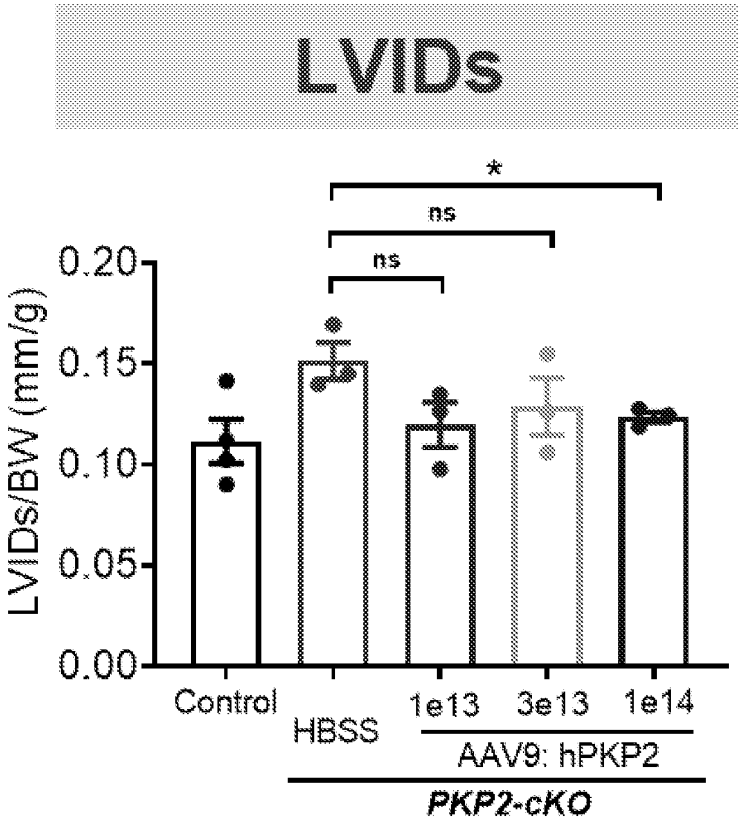

LVIDs
*
ns
ns
LVIDs/BW (mm/g)
0.20
0.15
0.10
0.05
0.00
Control
HBSS
1e13
3e13
1e14
AAV9: hPKP2
PKP2-cKO

FIG. 24D

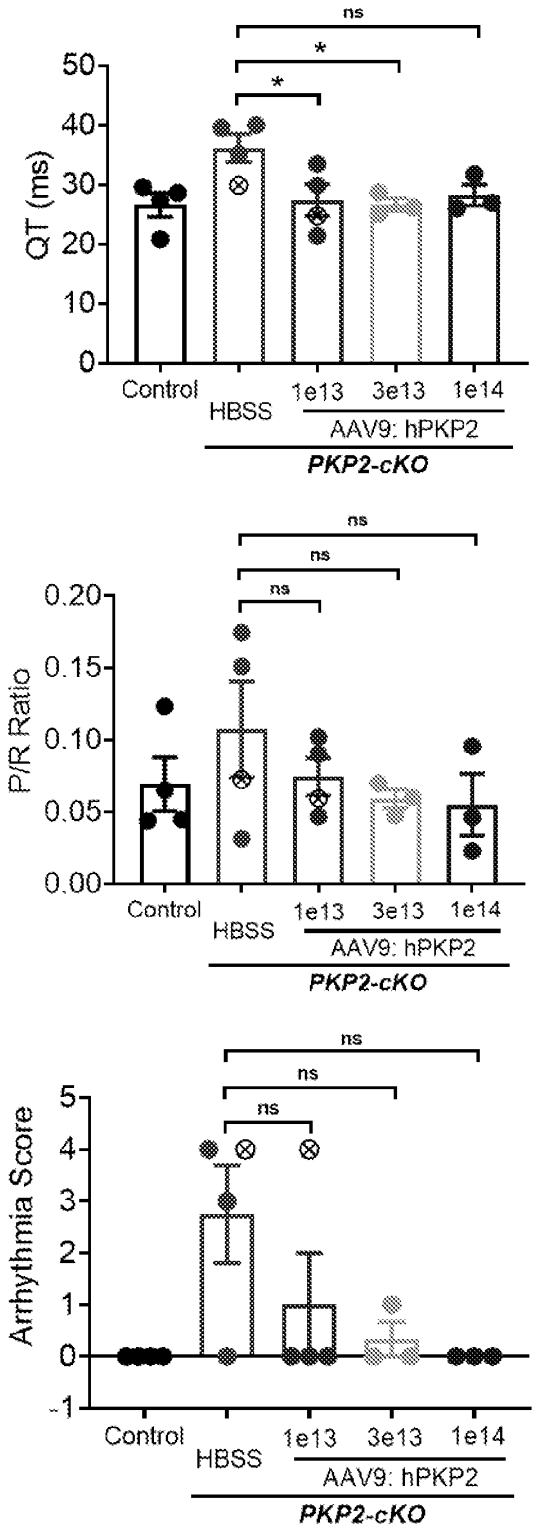

ns
*
*
50
40
30
20
10
0
QT (ms)
Control
HBSS
1e13
3e13
1e14
AAV9: hPKP2
PKP2-cKO
ns
ns
ns
0.20
0.15
0.10
0.05
0.00
P/R Ratio
Control
HBSS
1e13
3e13
1e14
AAV9: hPKP2
PKP2-cKO
ns
ns
ns
5
4
3
2
1
0
-1
Arrhythmia Score
Control
HBSS
1e13
3e13
1e14
AAV9: hPKP2
PKP2-cKO

FIG. 25

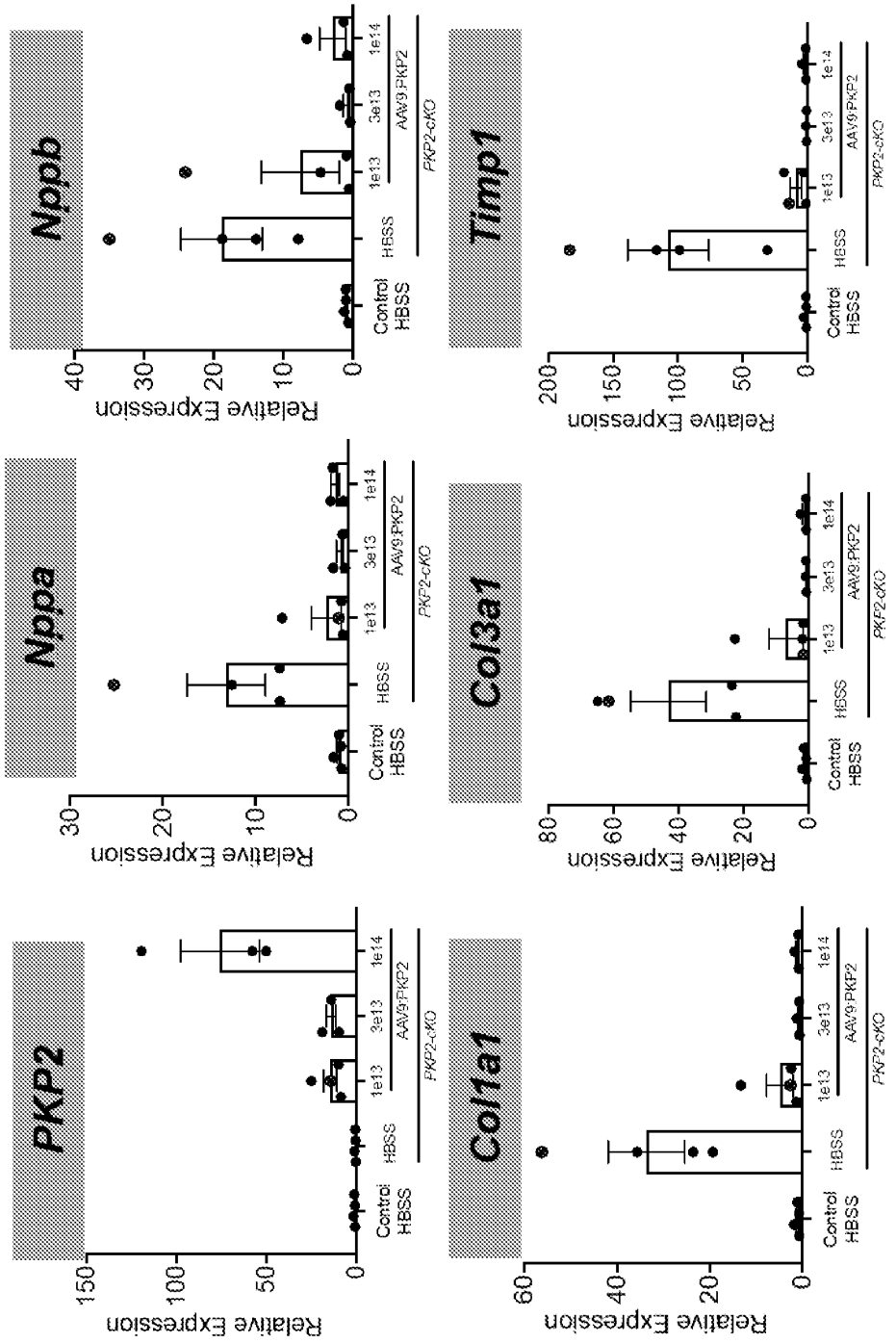

PKP2
Nppa
Nppb
Col1a1
Col3a1
Timp1
Relative Expression
Control
HBSS
HBSS
1e13
3e13
1e14
AAV9-PKP2
PKP2-cKO

FIG. 26A

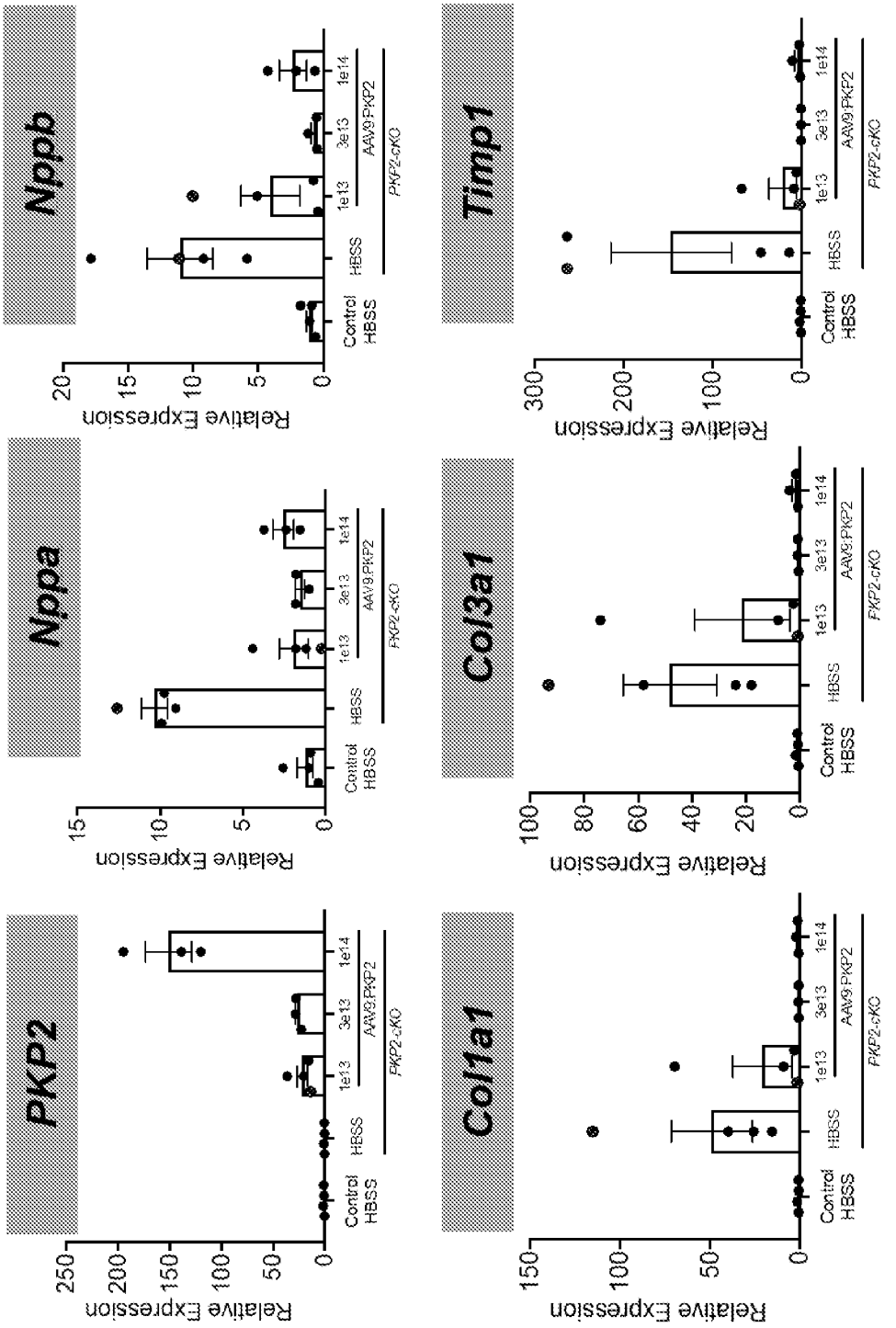

Nppb
Nppa
Timp1
PKP2
Col3a1
Col1a1
Relative Expression
Control
HBSS
HBSS
1e13
3e13
1e14
AAV9:PKP2
PKP2-cKO

FIG. 26B

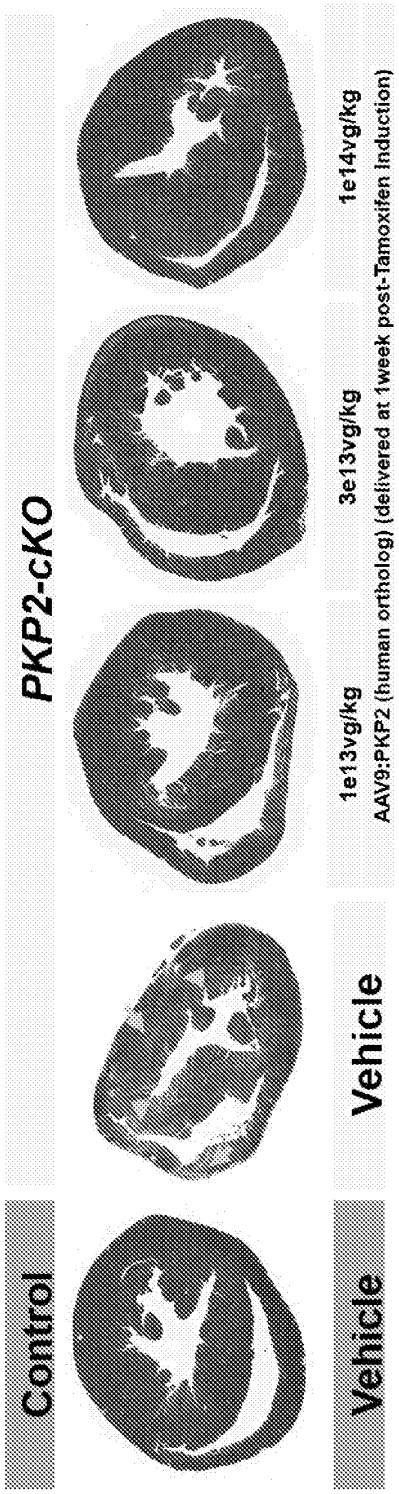

Control
Vehicle
PKP2-cKO
Vehicle
1e13vg/kg
3e13vg/kg
1e14vg/kg
AAV9:PKP2 (human ortholog) (delivered at 1week post-Tamoxifen induction)

FIG. 27A

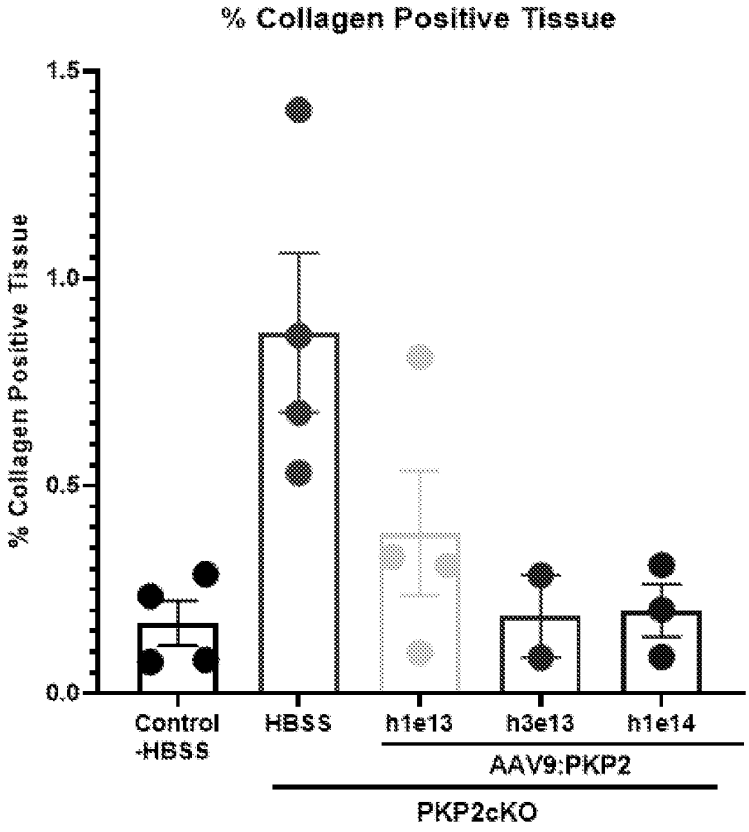

% Collagen Positive Tissue
% Collagen Positive Tissue
1.5
1.0
0.5
0.0
Control -HBSS
HBSS
h1e13
h3e13
h1e14
AAV9:PKP2
PKP2cKO

FIG. 27B

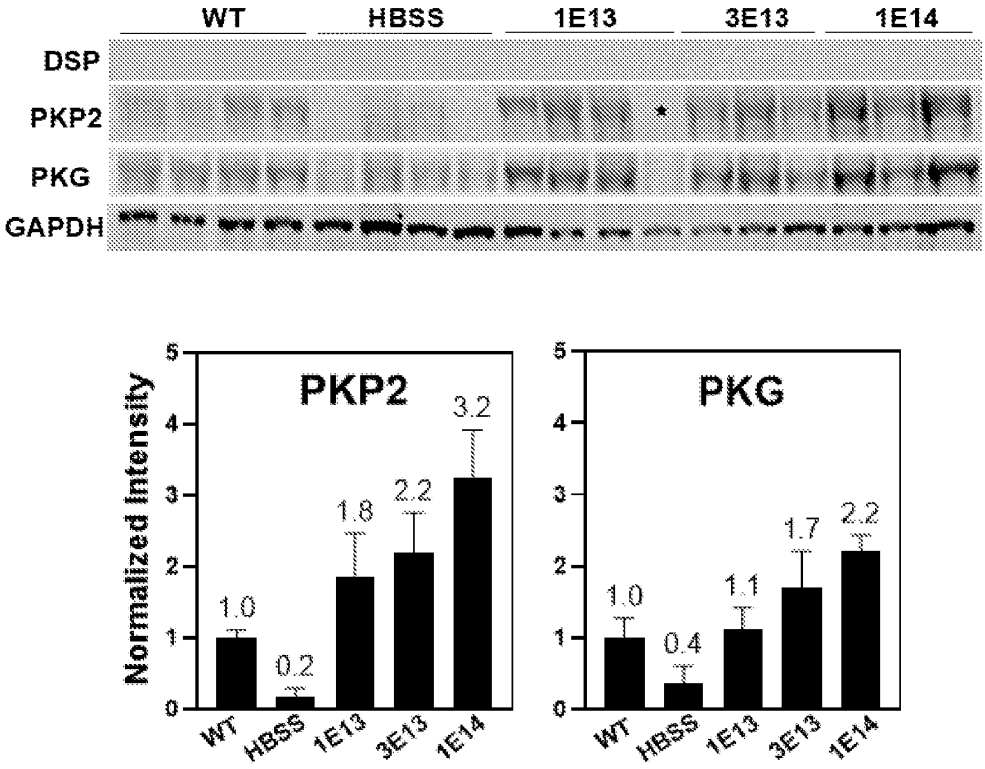

WT
HBSS
1E13
3E13
1E14
DSP
PKP2
PKG
GAPDH
Normalized Intensity
PKP2
PKG
1.0
0.2
1.8
2.2
3.2
1.0
0.4
1.1
1.7
2.2
0
1
2
3
4
5
WT
HBSS
1E13
3E13
1E14

FIG. 28A

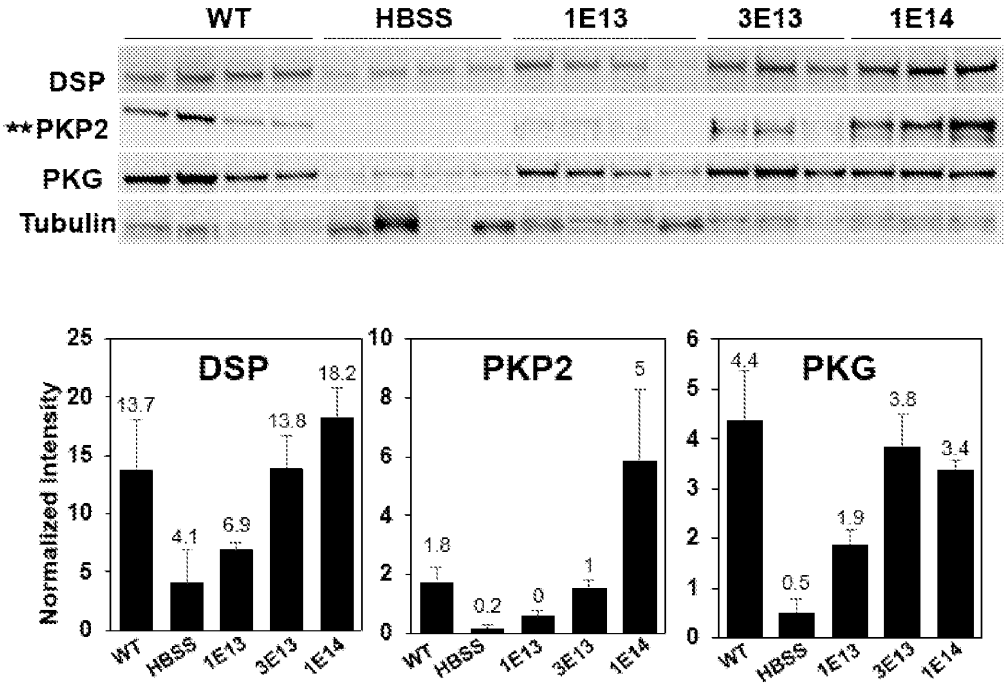

WT
HBSS
1E13
3E13
1E14
DSP
**PKP2
PKG
Tubulin
Normalized intensity
DSP
PKP2
PKG
13.7
4.1
6.9
13.8
18.2
1.8
0.2
0
1
5
4.4
0.5
1.9
3.8
3.4

FIG. 28B