CaMKII and Arrhythmia

고신의대

차 태준

THE MOLECULAR BASIS OF CaMKII FUNCTION IN SYNAPTIC AND BEHAVIOURAL MEMORY

John Lisman, Howard Schulman‡ and Hollis Cline§*

Long-term potentiation (LTP) in the CA1 region of the hippocampus has been the primary model by which to study the cellular and molecular basis of memory. Calcium/calmodulin-dependent protein kinase II (CaMKII) is necessary for LTP induction, is persistently activated by stimuli that elicit LTP, and can, by itself, enhance the efficacy of synaptic transmission. The analysis of CaMKII autophosphorylation and dephosphorylation indicates that this kinase could serve as a molecular switch that is capable of long-term memory storage. Consistent with such a role, mutations that prevent persistent activation of CaMKII block LTP, experience-dependent plasticity and behavioural memory. These results make CaMKII a leading candidate in the search for the molecular basis of memory.

POSTSYNAPTIC DENSITY
An electron-dense thickening underneath the postsynaptic membrane at excitatory synapses that contains receptors, structural proteins linked to the actin cytoskeleton and signalling elements, such as kinases and phosphatases.

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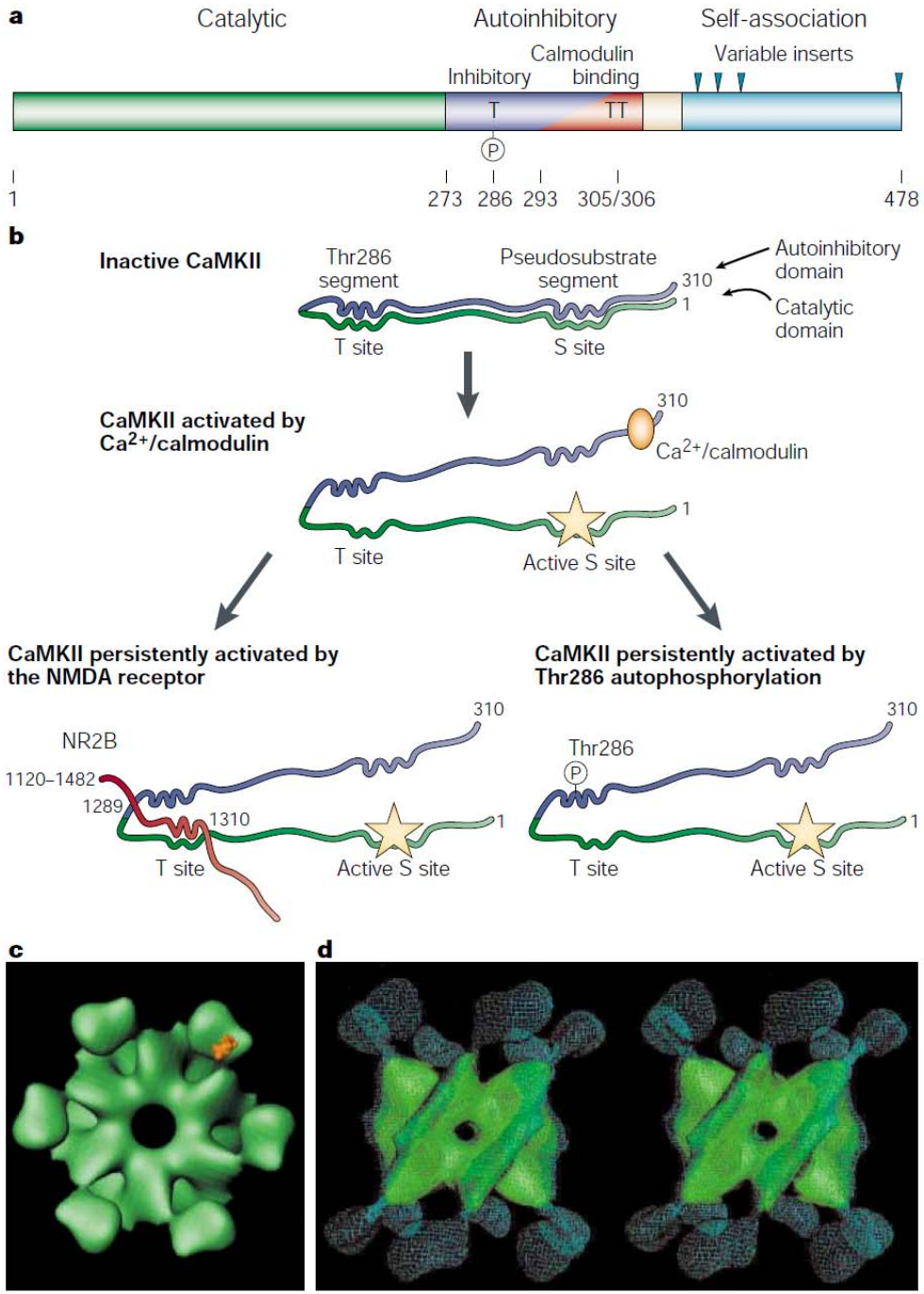
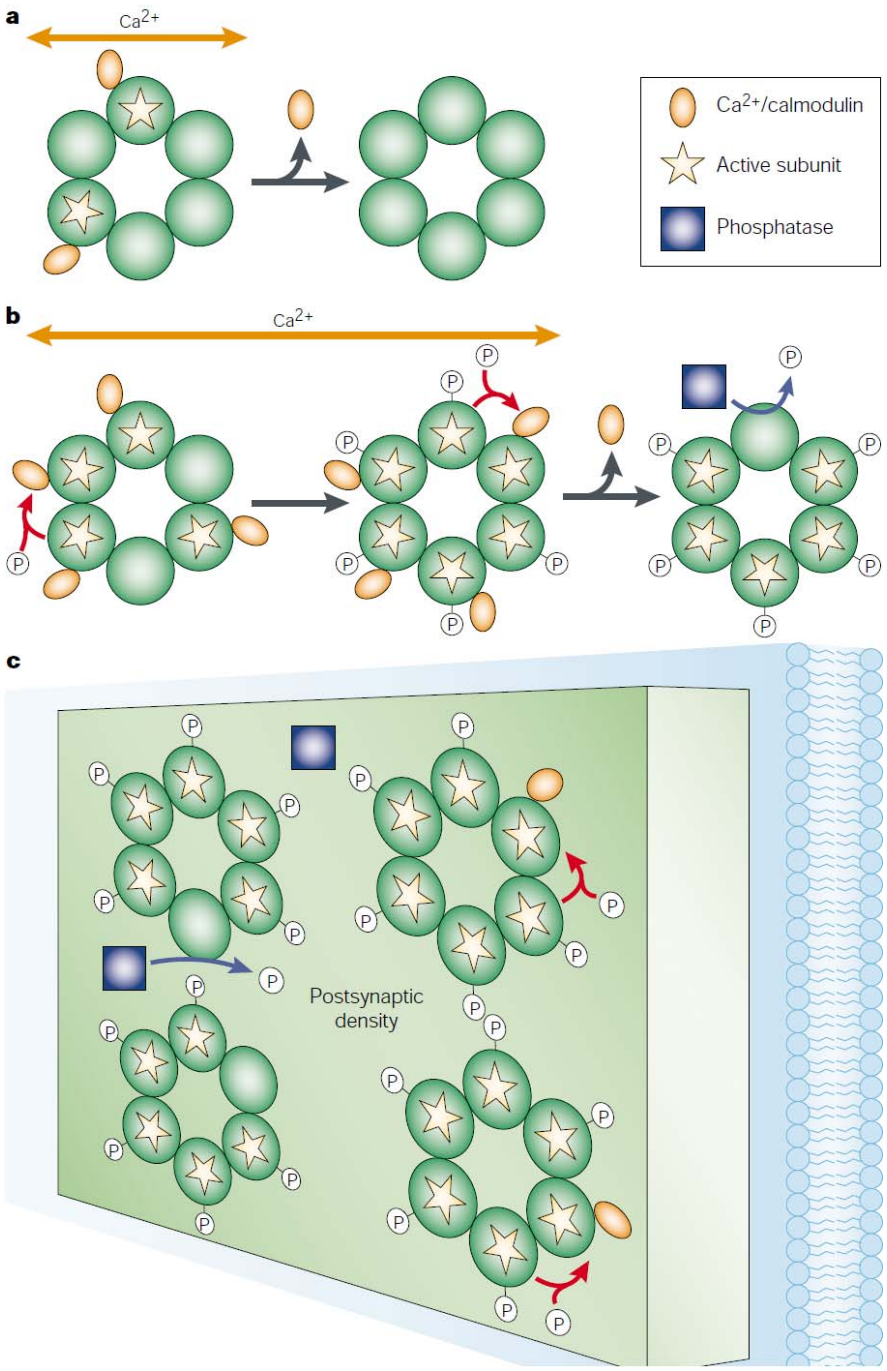
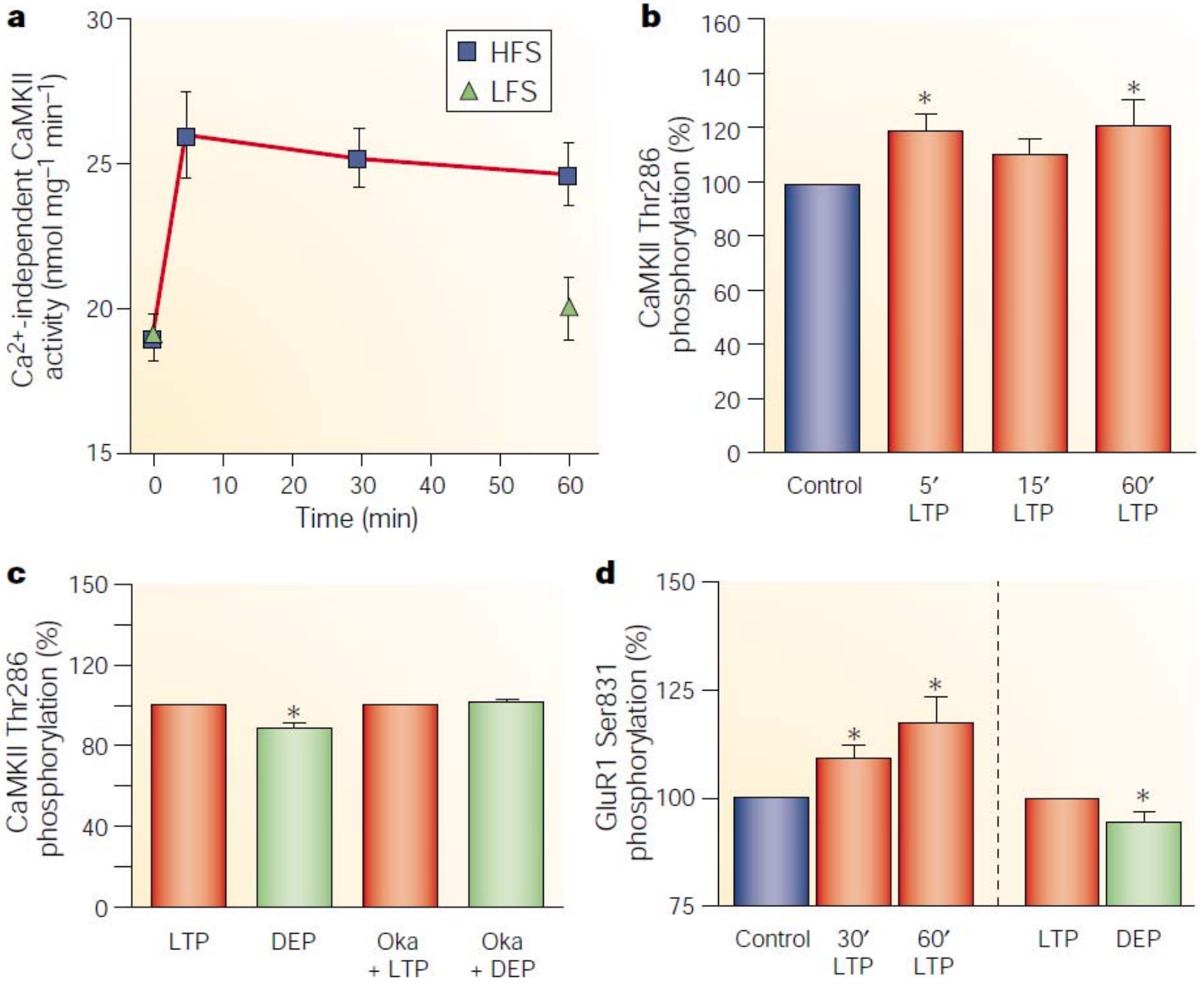
Calcium/calmodulin-dependent protein kinase II (CaMKII) is a Ca^{2+}-activated enzyme that is highly abundant in the brain, where it constitutes 1–2% of the total protein. The kinase is enriched at synapses and is the main protein of the POSTSYNAPTIC DENSITY (PSD) (FIG. 1). CaMKII is central to the regulation of glutamatergic synapses. This conclusion has emerged largely from the study of long-term potentiation (LTP), an activity-dependent strengthening of synapses that is thought to underlie some forms of learning and memory. At many excitatory synapses, LTP is triggered by Ca^{2+} entry into the postsynaptic cell. Several lines of evidence indicate that CaMKII detects this Ca^{2+} elevation and initiates the biochemical cascade that potentiates synaptic transmission.

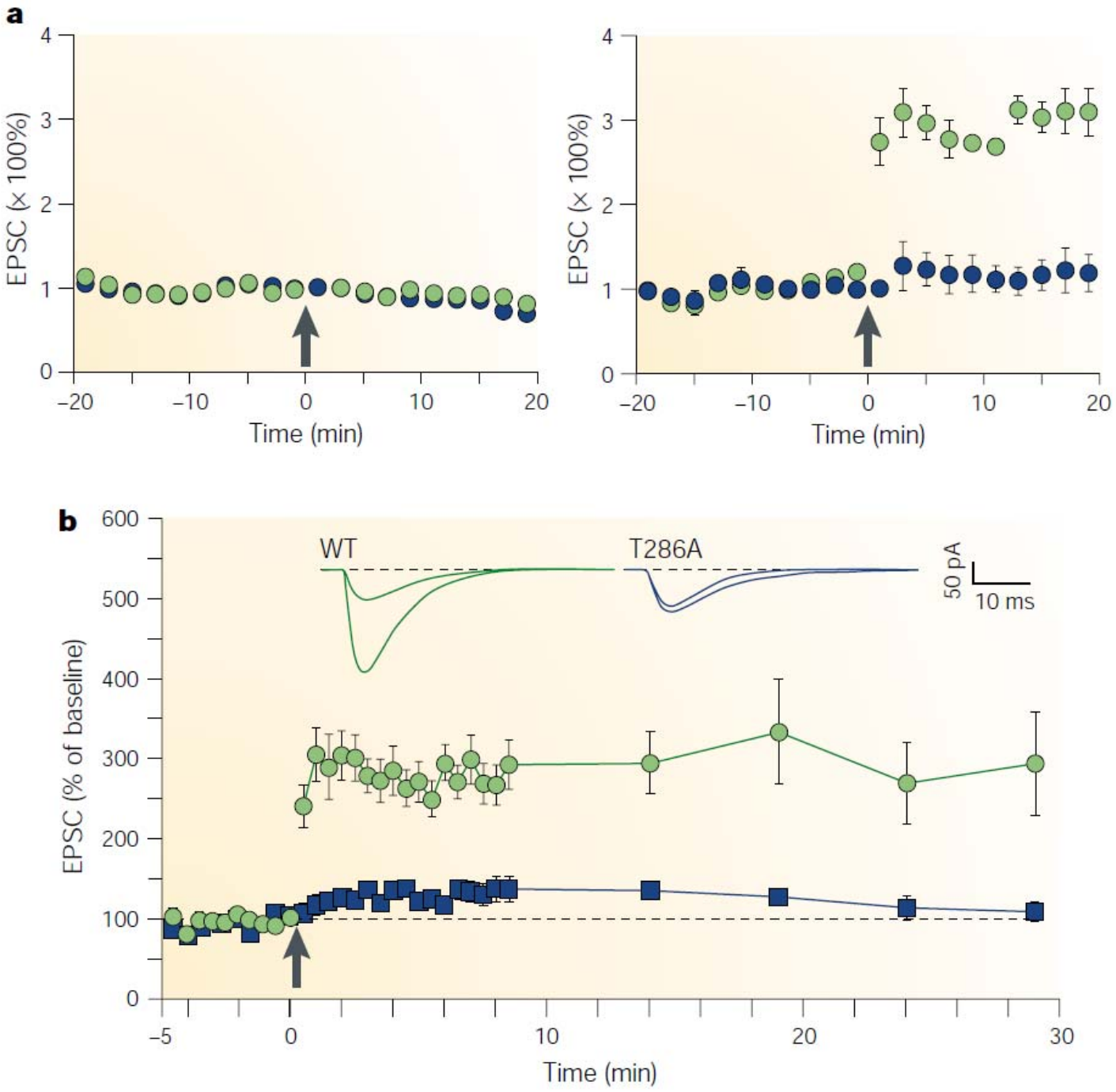
But CaMKII might function as more than just a transducer during LTP induction; the enzyme might also be directly responsible for the persistence of LTP and therefore have a memory function. The strongest evidence for this idea comes from the fact that CaMKII remains activated for at least one hour after LTP induction — the longest period examined so far. Furthermore, autophosphorylation of threonine 286 is crucial for its persistent activation; a mutation that eliminates phosphorylation of this site blocks LTP. Although these results show the importance of persistent activity, it remains to be established for how long this activity is required. Persistence of limited duration might suffice if information were passed on to another, more persistent downstream process. However, computational studies show that the persistent activity of CaMKII could be very long-lived, indicating that it could serve as a molecular basis of long-term synaptic memory without any downstream process.

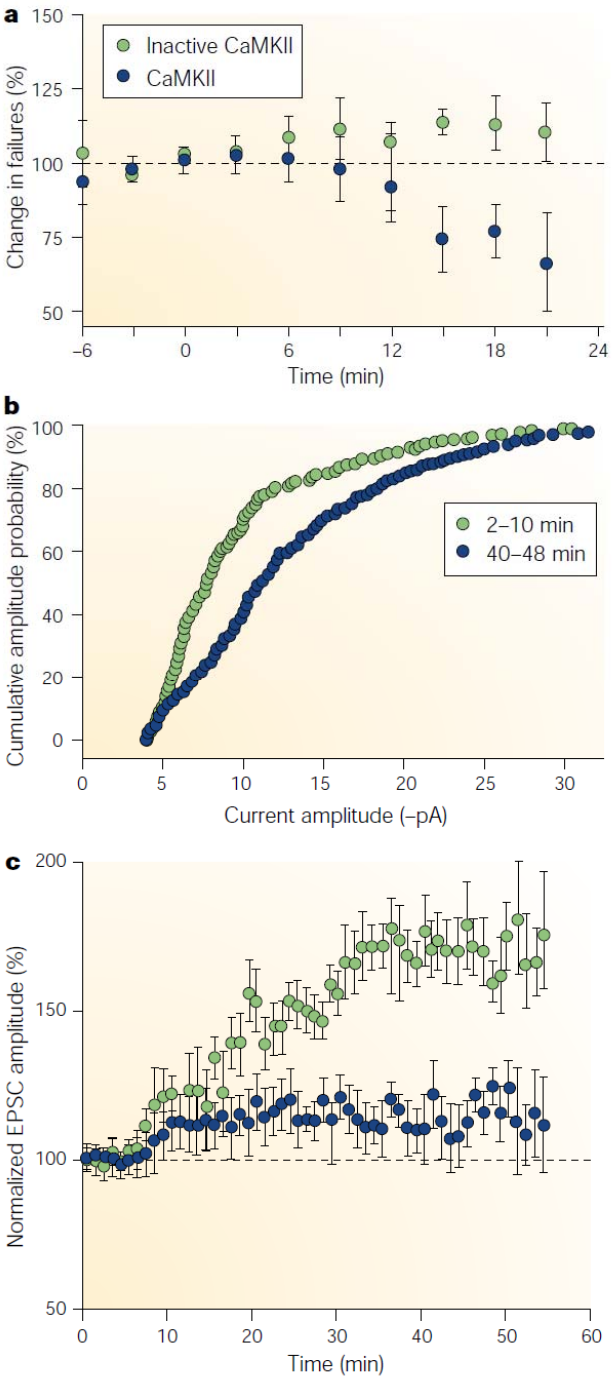
Progress in understanding the role of CaMKII has taken place at several levels. At the molecular level, there is now a better understanding of how autophosphorylation leads to persistent activity. Furthermore, recent studies show that CaMKII translocates to synapses, where it binds directly to the NMDA (*N*-methyl-D-aspartate) receptor. This translocation places the kinase in an ideal site to control synaptic strength; the molecular and structural processes by which this strengthening occurs are beginning to be unravelled. Progress has also been made in understanding how CaMKII contributes to brain function at the systems level. This is best exemplified by the observation that eliminating Thr286 phosphorylation not only blocks LTP, but also interferes


a
Catalytic
Autoinhibitory
Self-association
Calmodulin binding
Inhibitory
Variable inserts
T
TT
P
1
273
286
293
305/306
478
b
Inactive CaMKII
Thr286 segment
Pseudosubstrate segment
310
1
Autoinhibitory domain
Catalytic domain
T site
S site
CaMKII activated by Ca2+/calmodulin
310
Ca2+/calmodulin
1
T site
Active S site
CaMKII persistently activated by the NMDA receptor
CaMKII persistently activated by Thr286 autophosphorylation
NR2B
1120–1482
1289
1310
310
1
T site
Active S site
Thr286
P
310
1
T site
Active S site
c
d


a
Ca2+
Ca2+/calmodulin
Active subunit
Phosphatase
b
Ca2+
P
c
Postsynaptic
density


a
30
25
20
15
HFS
LFS
Ca2+-independent CaMKII activity (nmol mg−1 min−1)
0
10
20
30
40
50
60
Time (min)
b
160
140
120
100
80
60
40
20
0
CaMKII Thr286 phosphorylation (%)
*
*
Control
5′ LTP
15′ LTP
60′ LTP
c
150
120
80
40
0
CaMKII Thr286 phosphorylation (%)
*
LTP
DEP
Oka + LTP
Oka + DEP
d
150
125
100
75
GluR1 Ser831 phosphorylation (%)
*
*
*
Control
30′ LTP
60′ LTP
LTP
DEP


a
EPSC (× 100%)
Time (min)
b
WT
T286A
50 pA
10 ms
EPSC (% of baseline)
Time (min)


a
Inactive CaMKII
CaMKII
Change in failures (%)
150
125
100
75
50
–6
0
6
12
18
24
Time (min)
b
Cumulative amplitude probability (%)
100
80
60
40
20
0
2–10 min
40–48 min
0
5
10
15
20
25
30
Current amplitude (–pA)
c
Normalized EPSC amplitude (%)
200
150
100
50
0
10
20
30
40
50
60
Time (min)

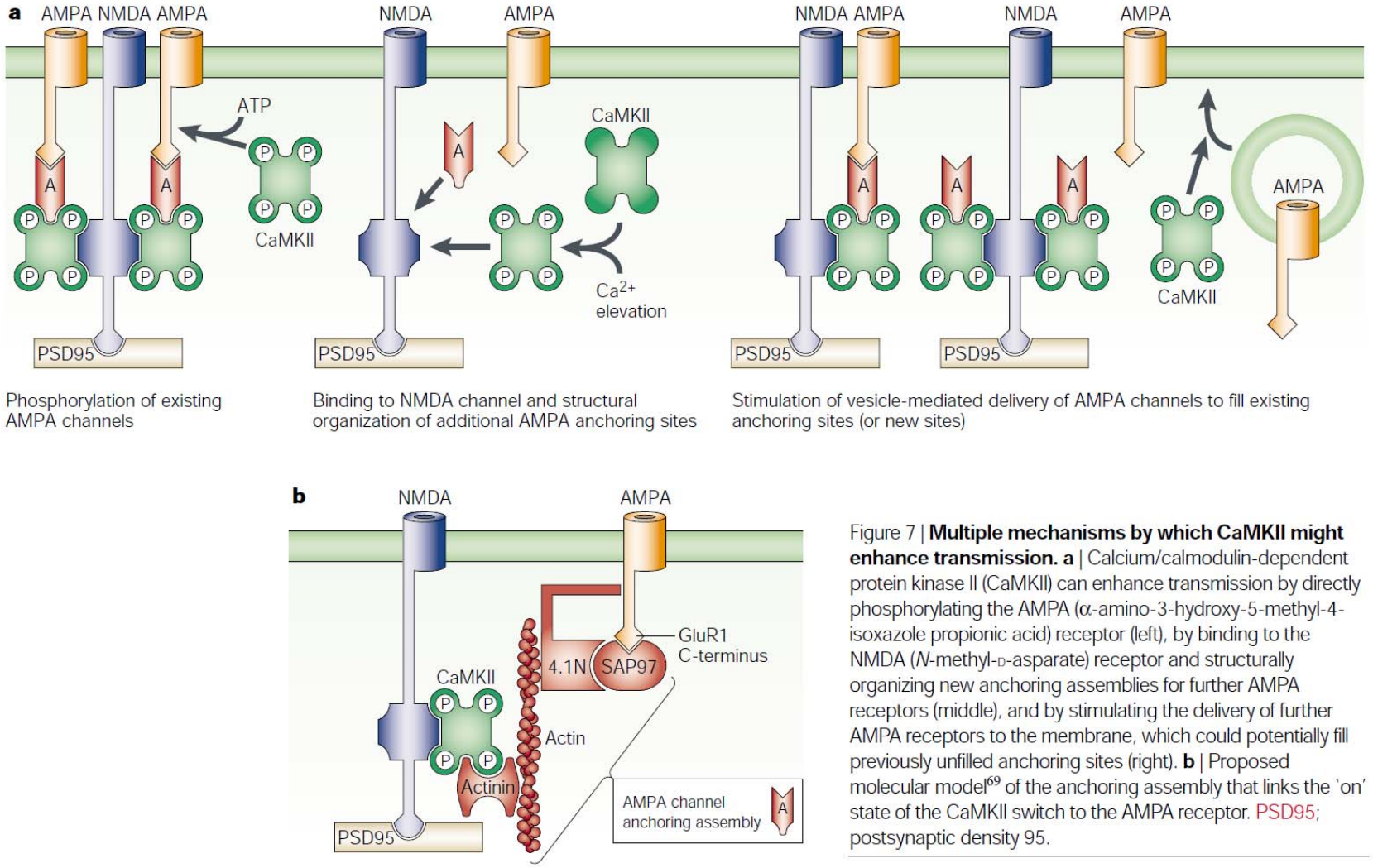
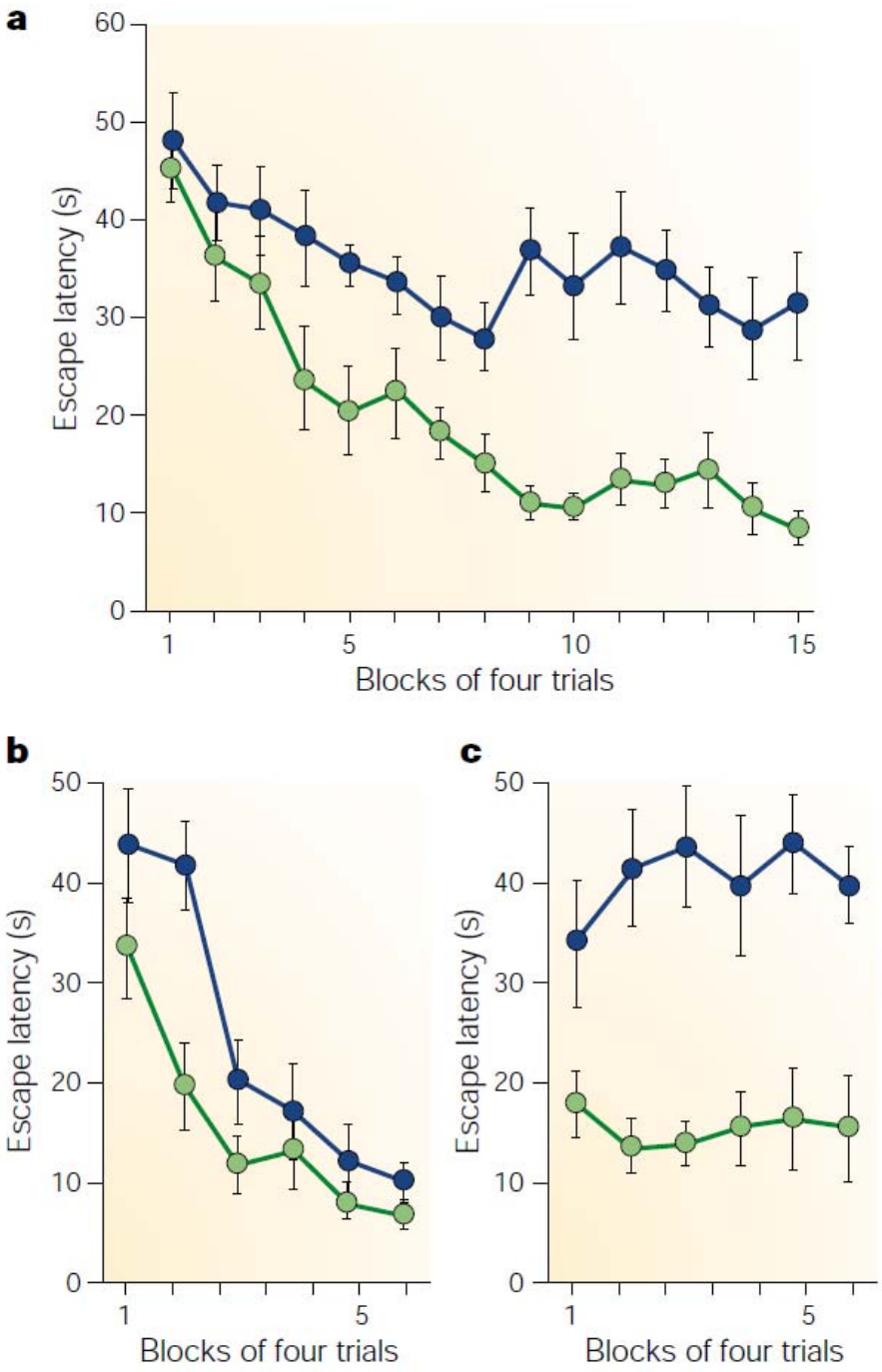
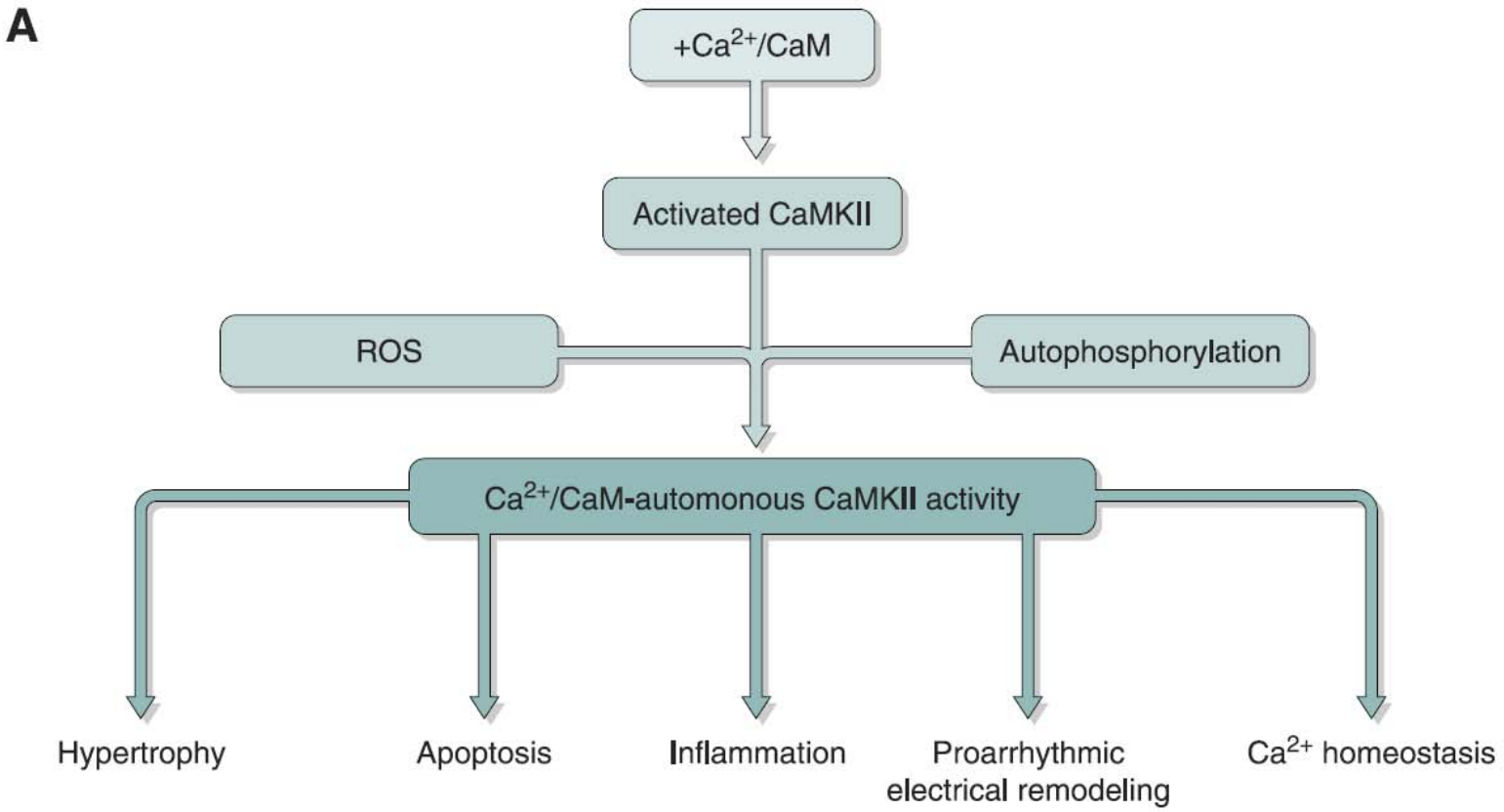


Figure 7 | **Multiple mechanisms by which CaMKII might enhance transmission. a** | Calcium/calmodulin-dependent protein kinase II (CaMKII) can enhance transmission by directly phosphorylating the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor (left), by binding to the NMDA (*N*-methyl-D-asparate) receptor and structurally organizing new anchoring assemblies for further AMPA receptors (middle), and by stimulating the delivery of further AMPA receptors to the membrane, which could potentially fill previously unfilled anchoring sites (right). **b** | Proposed molecular model[69] of the anchoring assembly that links the 'on' state of the CaMKII switch to the AMPA receptor. PSD95; postsynaptic density 95.


a
Escape latency (s)
60
50
40
30
20
10
0
1
5
10
15
Blocks of four trials
b
Escape latency (s)
50
40
30
20
10
0
1
5
Blocks of four trials
c
Escape latency (s)
50
40
30
20
10
0
1
5
Blocks of four trials

- **The multifunctional Ca^{2+}- and calmodulin-dependent protein kinase II (CaMKII) is a serine/threonine kinase that modulates each of these biological functions in diverse cell types.**


A
+Ca2+/CaM
Activated CaMKII
ROS
Autophosphorylation
Ca2+/CaM-automonous CaMKII activity
Hypertrophy
Apoptosis
Inflammation
Proarrhythmic electrical remodeling
Ca2+ homeostasis

Calcium signaling in cardiac gene expression and hypertrophic growth

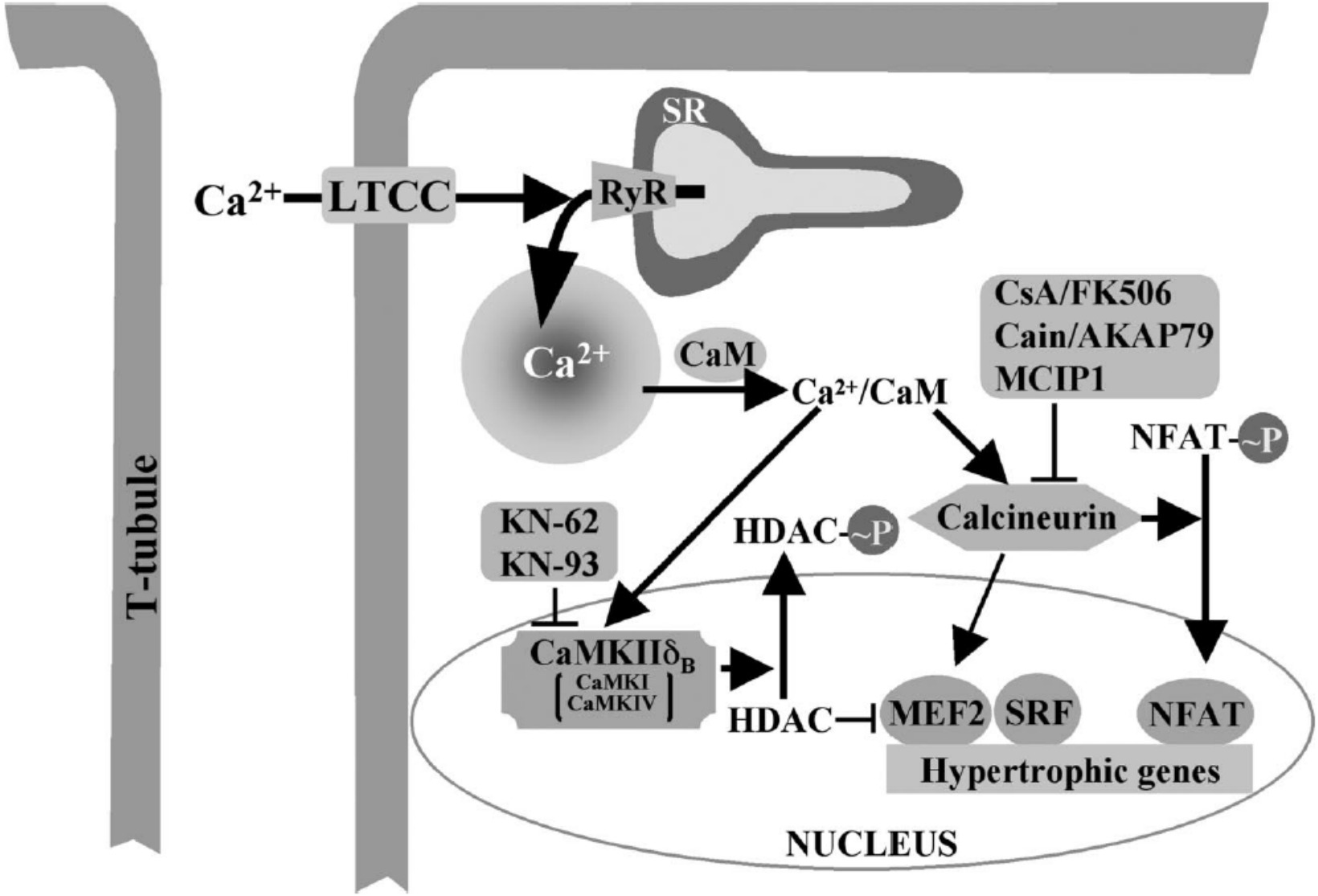
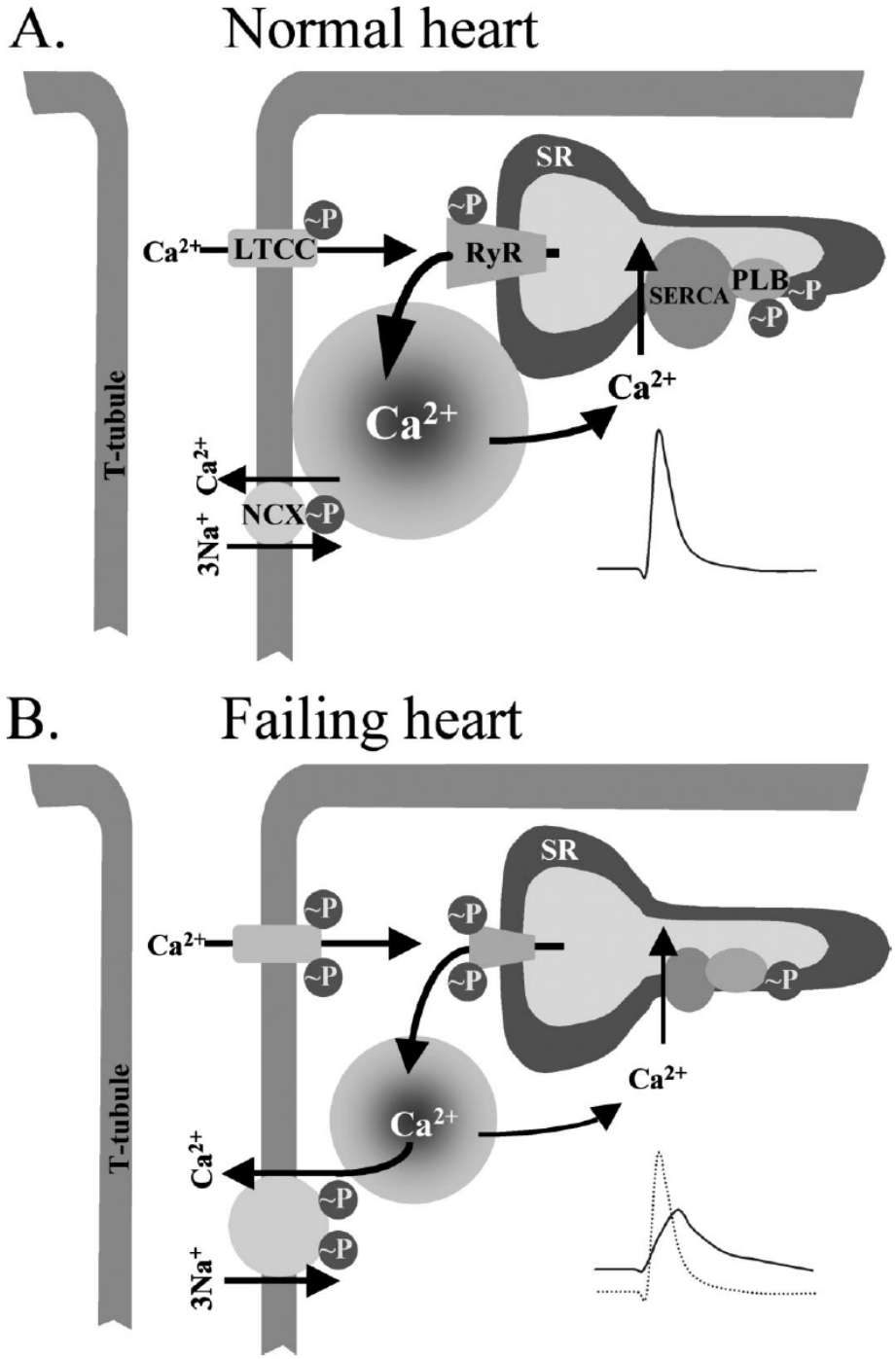


TABLE I

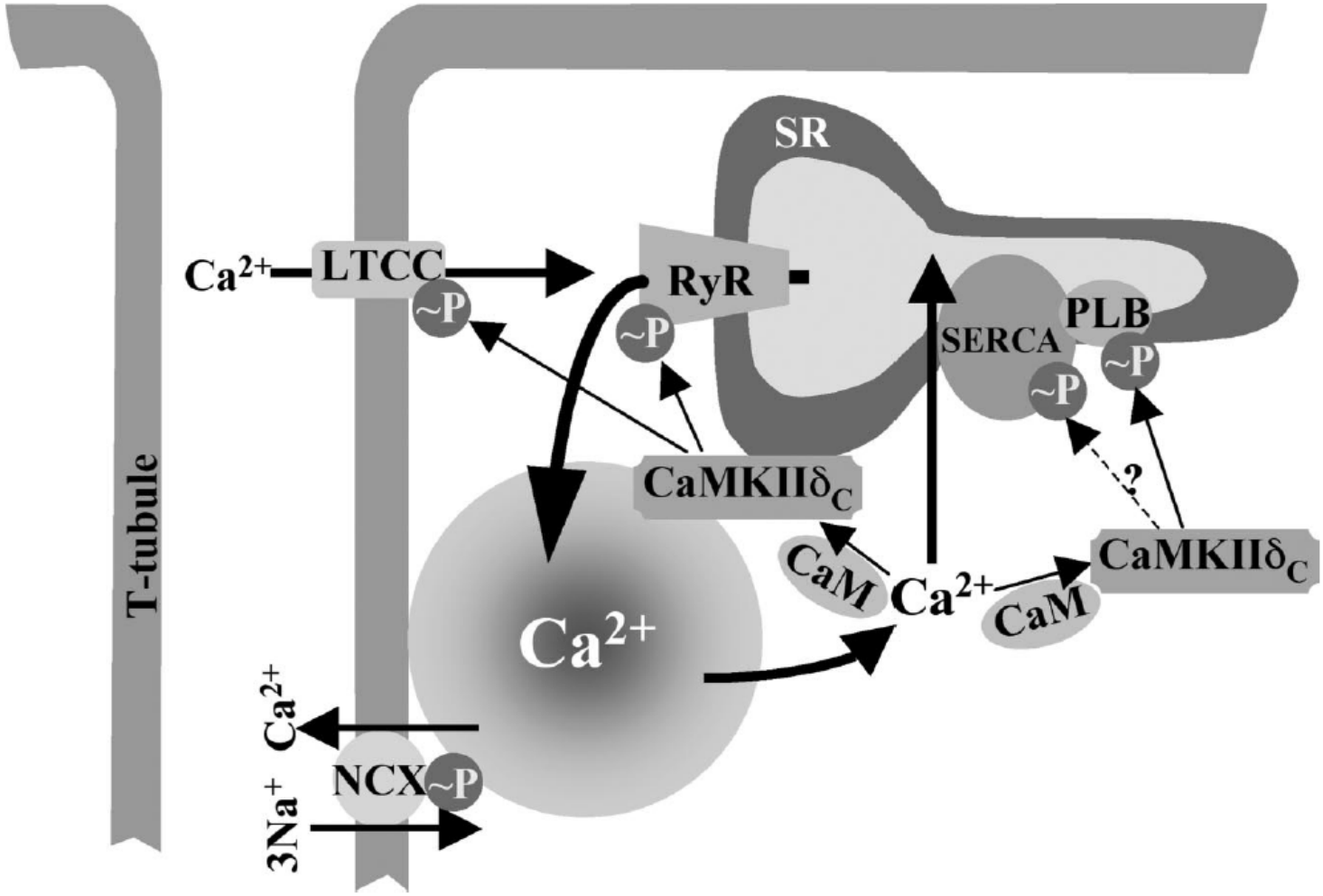
Summary of Animal Models Showing Ca^{2+}/Calmodulin-dependent Protein Kinase (CaMK) Involvement in Cardiac Hypertrophy

Animal model	Phenotype and effects	References
Hypertensive rat models	Cardiac hypertrophy and increased CaMKII expression	Hagemann *et al.*, 2001
Spontaneously hypertensive rats	Cardiac hypertrophy and increased CaMKII activity	Boknik *et al.*, 2001
Coronary artery ligation rabbit	Cardiac hypertrophy and increased CaMKII activity	Currie *et al.*, 1999
Transverse aortic constricted mice	Cardiac hypertrophy and increased CaMKII expression and activity	Colomer *et al.*, 2003; Zhang *et al.*, 2003
Calmodulin TG mice	Severe cardiac hypertrophy and increased CaMKII activity	Gruver *et al.*, 1993; Colomer *et al.*, 2000
CaMKIV TG mice	Cardiac hypertrophy through MEF2 activation	Passier *et al.*, 2000
CaMKIIδ_B TG mice	Cardiac hypertrophy and dilated cardiomyopathy	Zhang *et al.*, 2002

Abbreviations: TG, transgenic; MEF, myocyte enhancer factor.


A.
Normal heart
SR
~P
~P
Ca2+
LTCC
RyR
SERCA
PLB
~P
~P
T-tubule
Ca2+
Ca2+
Ca2+
3Na+
NCX
~P
B.
Failing heart
SR
~P
~P
~P
~P
~P
Ca2+
Ca2+
Ca2+
Ca2+
~P
~P
3Na+

Regulation of cardiomyocyte Calcium homeostasis by CaMKII



Calmodulin Kinase II and Arrhythmias in a Mouse Model of Cardiac Hypertrophy

Yuejin Wu, PhD*; Joel Temple, MD*; Rong Zhang, MD, PhD*; Igor Dzhura, PhD; Wei Zhang, MD; Robert Trimble, BS; Dan M. Roden, MD; Robert Passier, PhD; Eric N. Olson, PhD; Roger J. Colbran, PhD; Mark E. Anderson, MD, PhD

Background—Calmodulin kinase (CaMK) II is linked to arrhythmia mechanisms in cellular models where repolarization is prolonged. CaMKII upregulation and prolonged repolarization are general features of cardiomyopathy, but the role of CaMKII in arrhythmias in cardiomyopathy is unknown.

Methods and Results—We studied a mouse model of cardiac hypertrophy attributable to transgenic (TG) overexpression of a constitutively active form of CaMKIV that also has increased endogenous CaMKII activity. ECG-telemetered TG mice had significantly more arrhythmias than wild-type (WT) littermate controls at baseline, and arrhythmias were additionally increased by isoproterenol. Arrhythmias were significantly suppressed by an inhibitory agent targeting endogenous CaMKII. TG mice had longer QT intervals and action potential durations than WT mice, and TG cardiomyocytes had frequent early afterdepolarizations (EADs), a hypothesized mechanism for triggering arrhythmias. EADs were absent in WT cells before and after isoproterenol, whereas EAD frequency was unaffected by isoproterenol in TG mice. L-type Ca^{2+} channels (LTTCs) can activate EADs, and LTCC opening probability (Po) was significantly higher in TG than WT cardiomyocytes before and after isoproterenol. A CaMKII inhibitory peptide equalized TG and WT LTCC Po and eliminated EADs, whereas a peptide antagonist of the Na^{+}/Ca^{2+} exchanger current, also hypothesized to support EADs, was ineffective.

Conclusions—These findings support the hypothesis that CaMKII is a proarrhythmic signaling molecule in cardiac hypertrophy in vivo. Cellular studies point to EADs as a triggering mechanism for arrhythmias but suggest that the increase in arrhythmias after β-adrenergic stimulation is independent of enhanced EAD frequency. **(*Circulation*. 2002; 106:1288-1293.)**

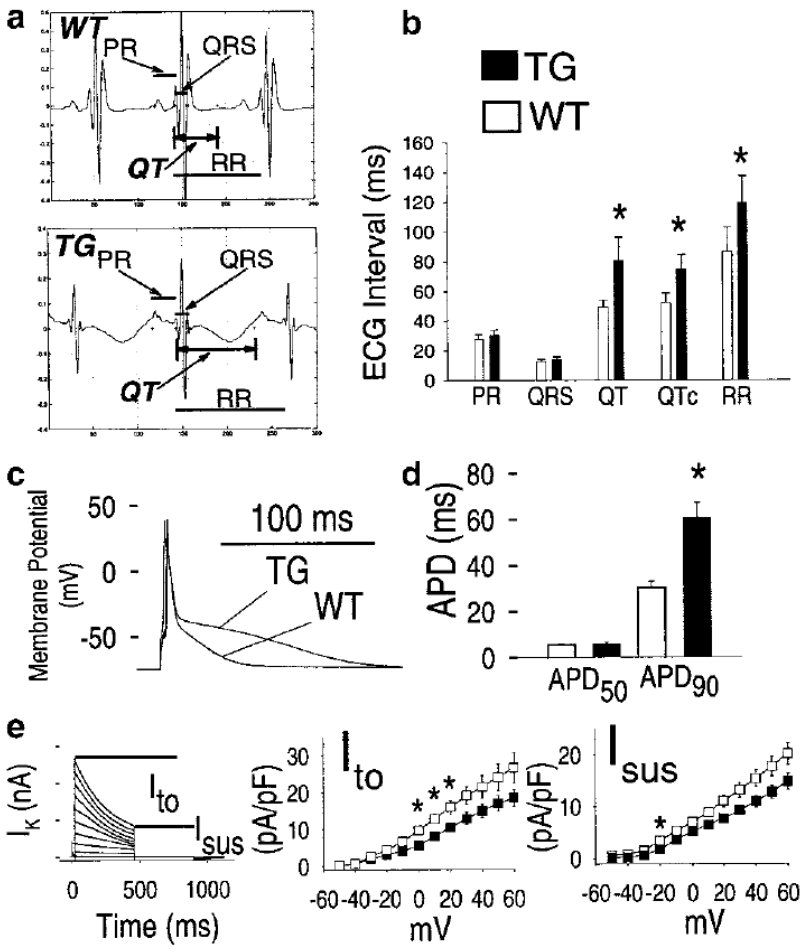
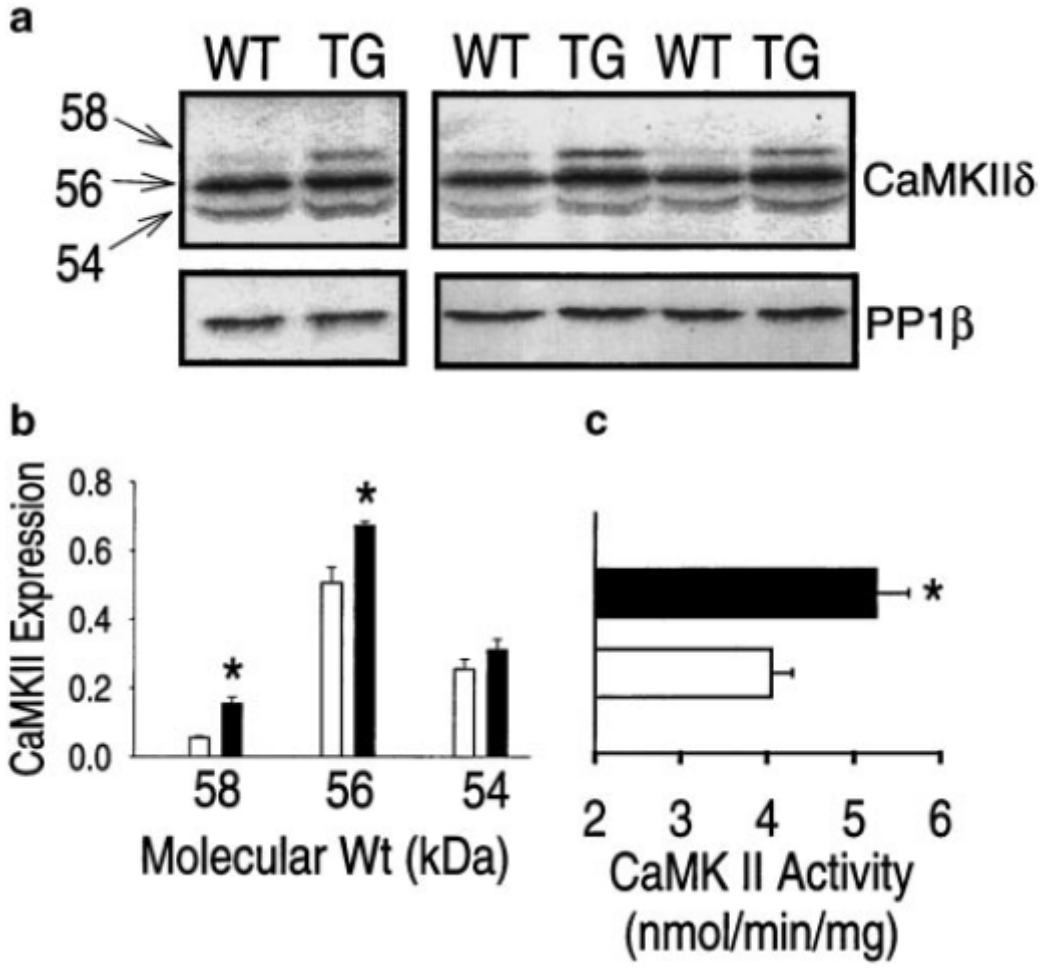
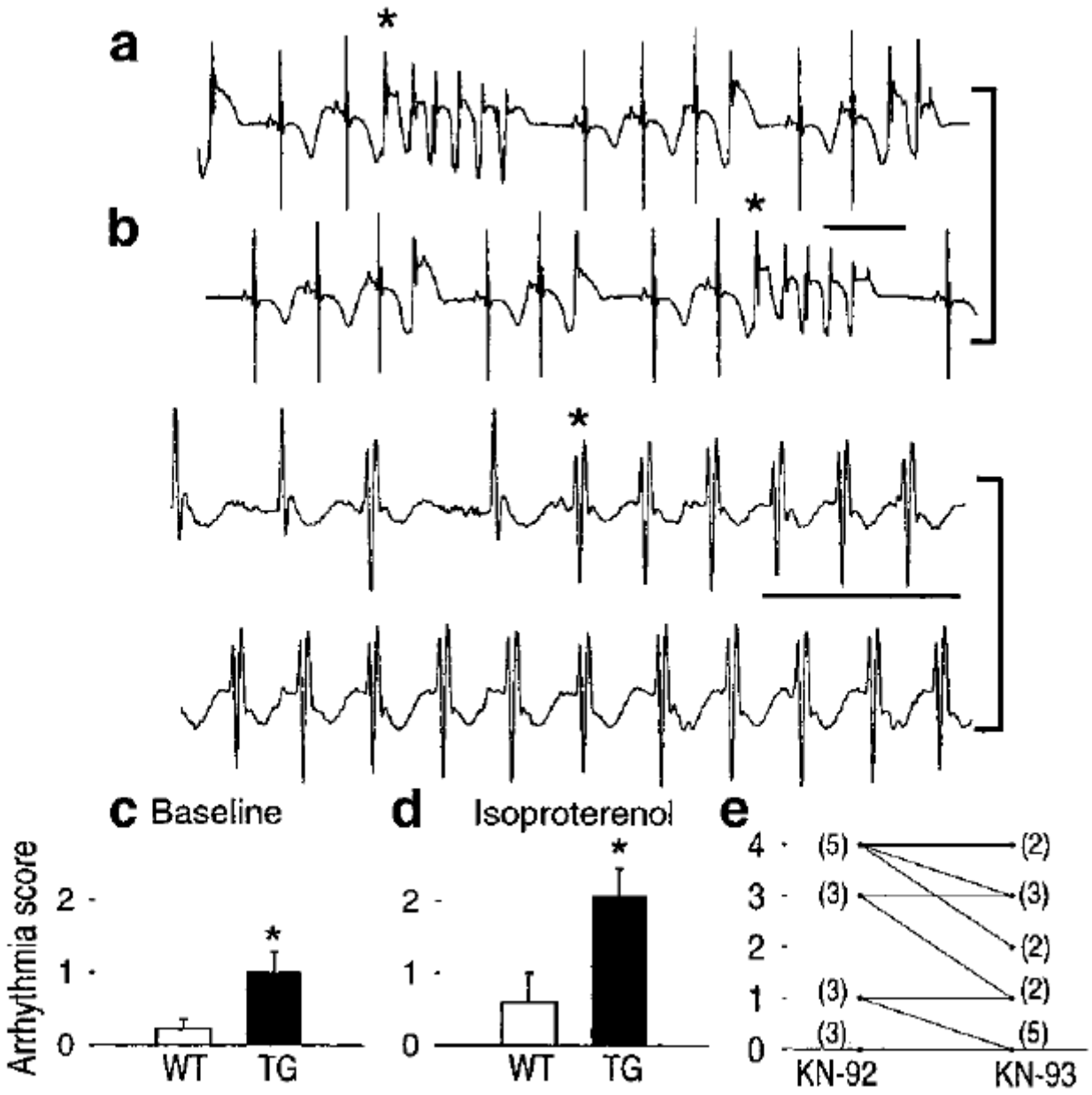
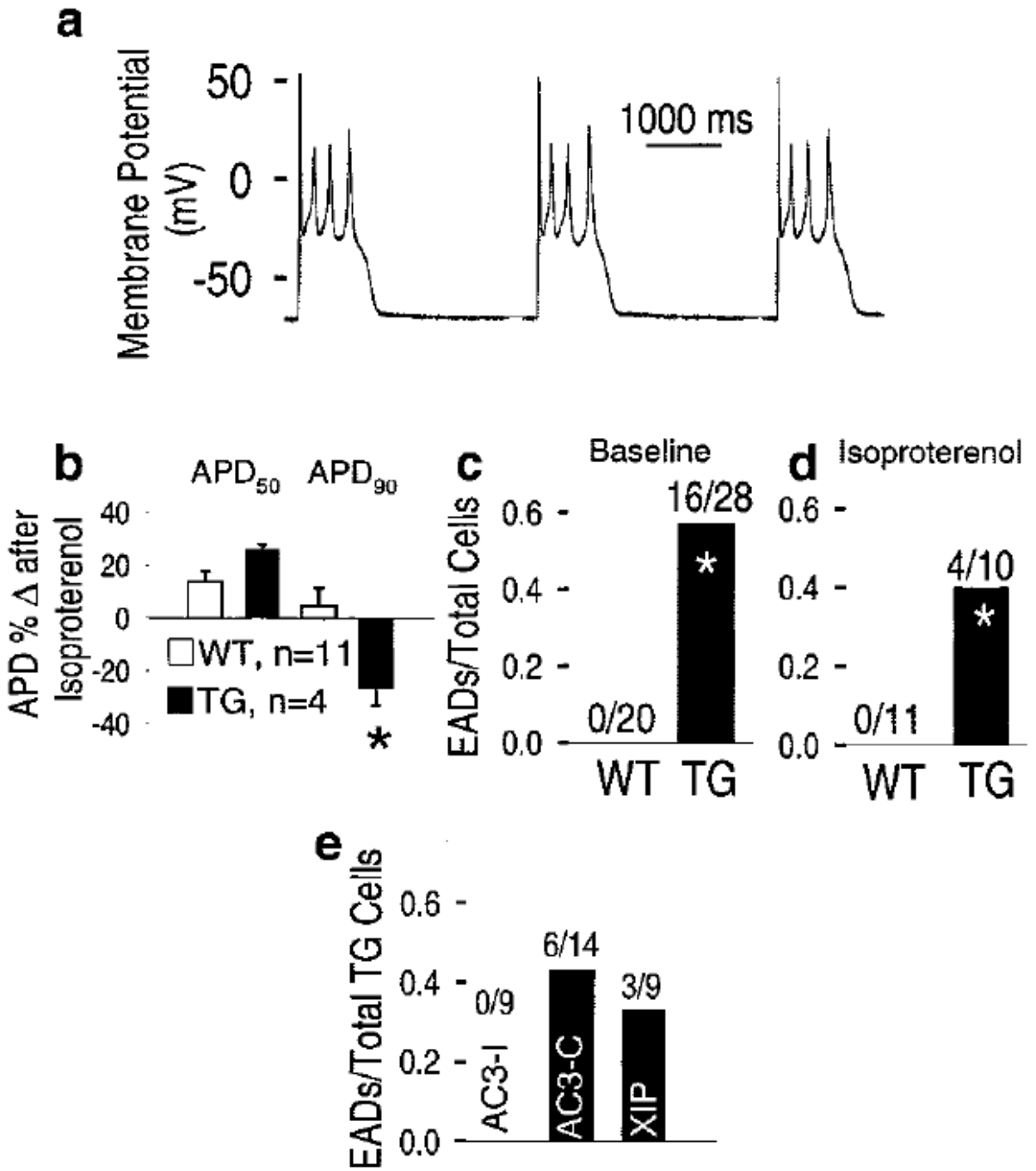
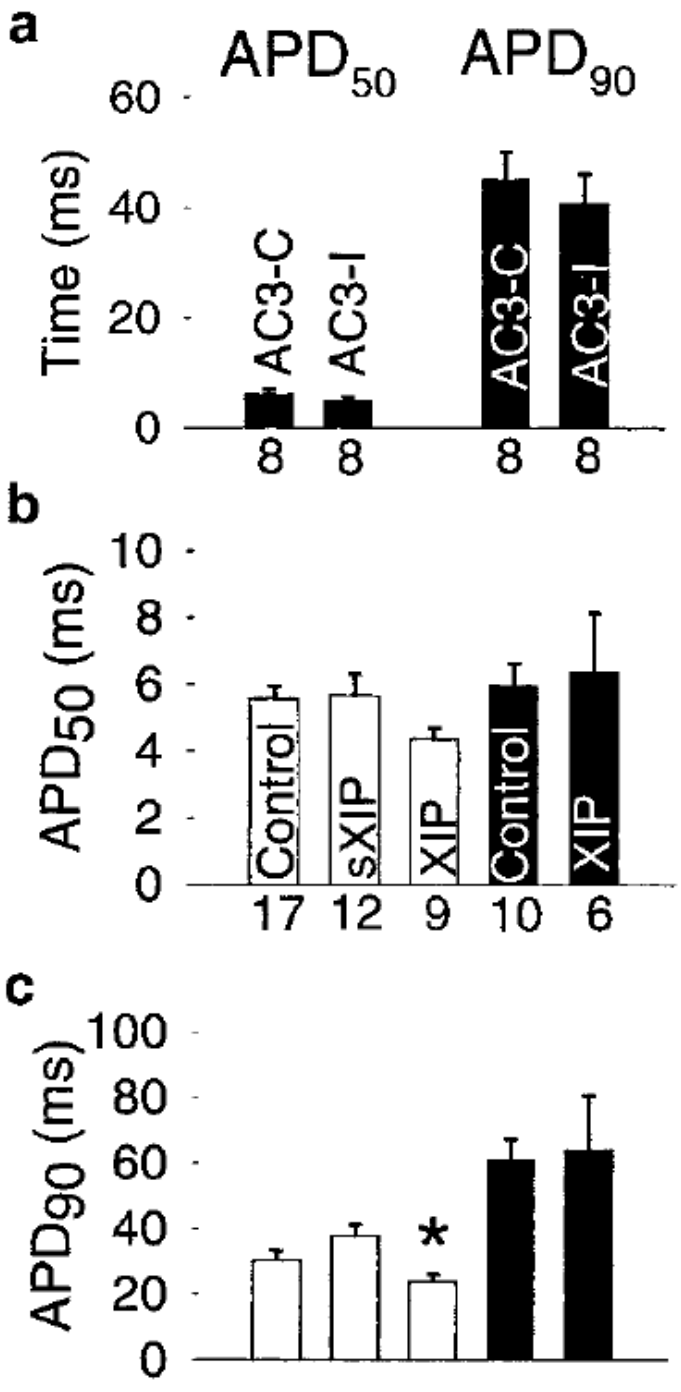


Figure 1. Electrical remodeling in TG mice. a, Signal-averaged ECGs show QT interval prolongation and abnormal QT interval displacement from the baseline in TG (bottom) compared with WT (top) mice. b, Summary ECG interval data from TG (n=8) and WT (n=6) mice. *$P<0.005$ for TG compared with WT mice for all ECG intervals. c, Superimposed action potential recordings show repolarization is prolonged in TG cardiomyocytes. d, Summary data for APD_{50} and APD_{90} repolarization in TG (n=10) compared with WT (n=17) cardiomyocytes. *$P<0.001$ for TG compared with WT. e, Transient (I_{to}, middle) and sustained (I_{sus}, right) components of repolarizing K^+ current are both reduced in TG (n=6) compared with WT (n=6) cardiomyocytes. Horizontal lines demarcate I_{to} and I_{sus} in this family of K^+ currents (left). *$P<0.05$ for I_{to} and I_{sus}.


a
WT TG WT TG WT TG
58
56
54
CaMKIIδ
PP1β
b
CaMKII Expression
0.8
0.6
0.4
0.2
0.0
*
*
58
56
54
Molecular Wt (kDa)
c
*
2 3 4 5 6
CaMK II Activity
(nmol/min/mg)


a
b
*
*
*
c Baseline
d Isoproterenol
e
Arrhythmia score
2
1
0
WT
TG
4
3
2
1
0
(5)
(3)
(3)
(3)
(2)
(3)
(2)
(2)
(5)
KN-92
KN-93


a
Membrane Potential (mV)
50
0
-50
1000 ms
b
APD50
APD90
APD % Δ after Isoproterenol
40
20
0
-20
-40
WT, n=11
TG, n=4
*
c
Baseline
EADs/Total Cells
0.6
0.4
0.2
0.0
16/28
*
0/20
WT
TG
d
Isoproterenol
0.6
0.4
0.2
0.0
4/10
*
0/11
WT
TG
e
EADs/Total TG Cells
0.6
0.4
0.2
0.0
0/9
AC3-I
6/14
AC3-C
3/9
XIP


a
APD50
APD90
60
40
20
0
Time (ms)
AC3-C
AC3-I
AC3-C
AC3-I
8
8
8
8
b
10
8
6
4
2
0
APD50 (ms)
Control
sXIP
XIP
Control
XIP
17
12
9
10
6
c
100
80
60
40
20
0
APD90 (ms)
*

Calmodulin kinase II is required for fight or flight sinoatrial node physiology

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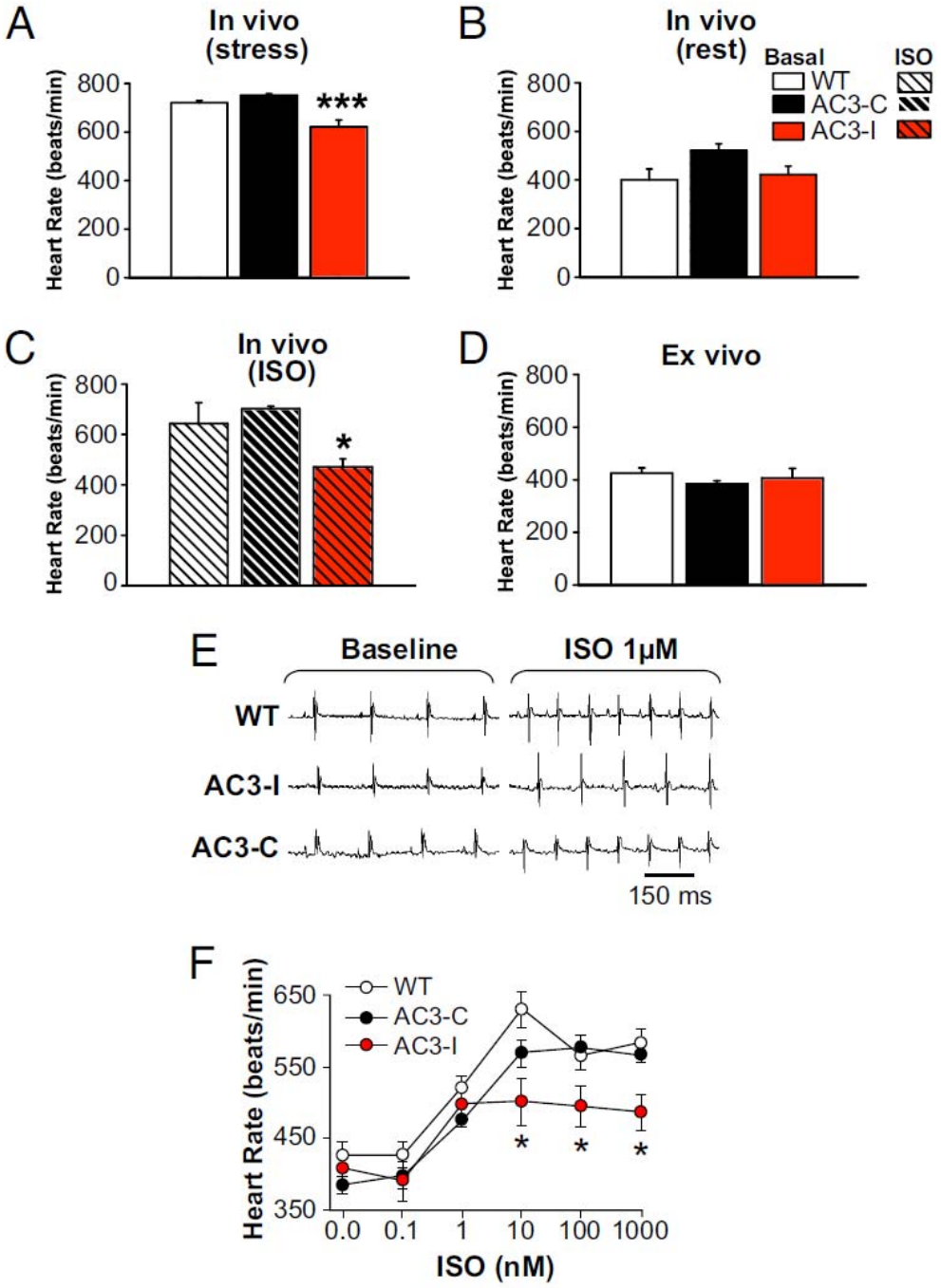
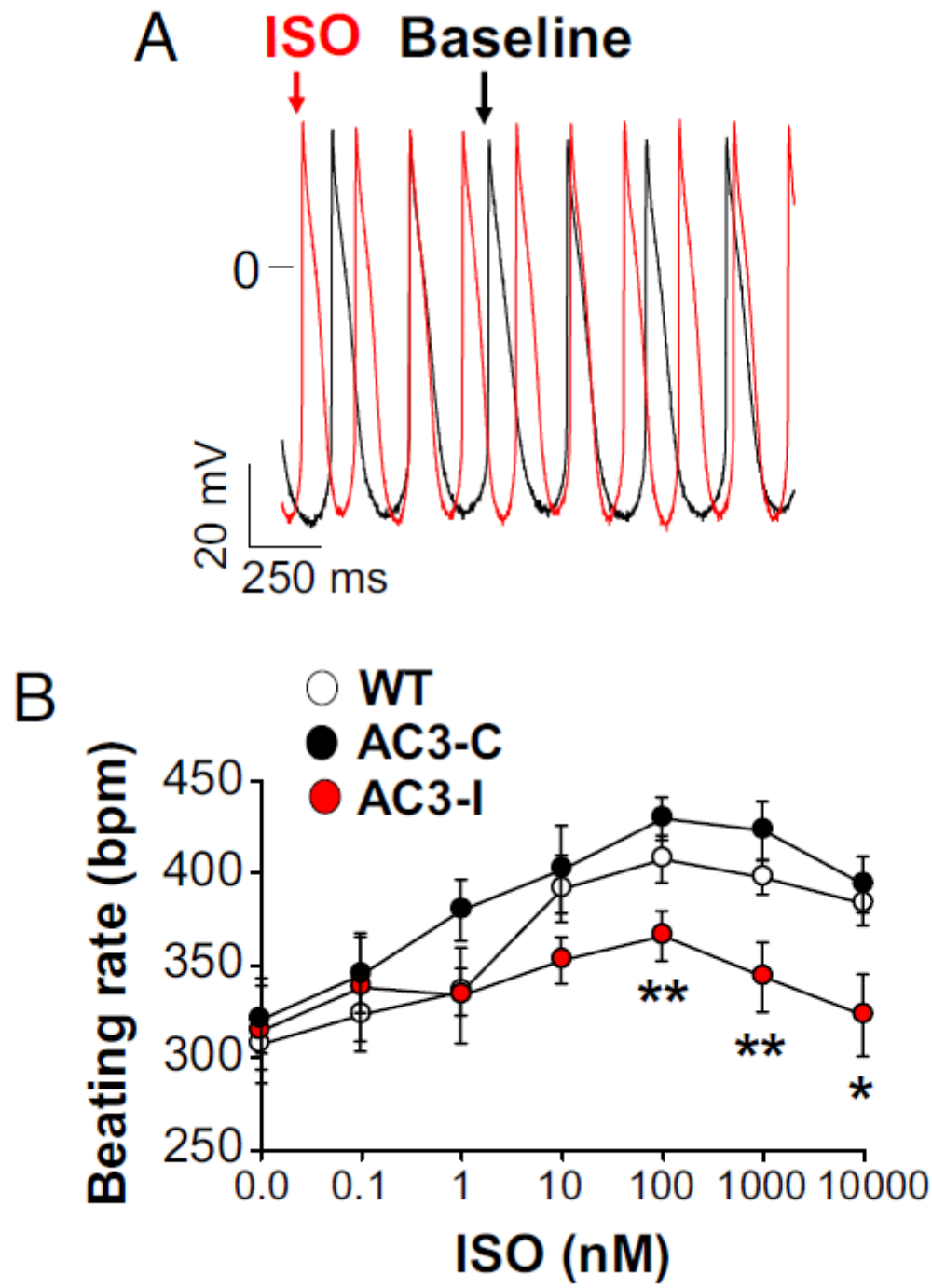
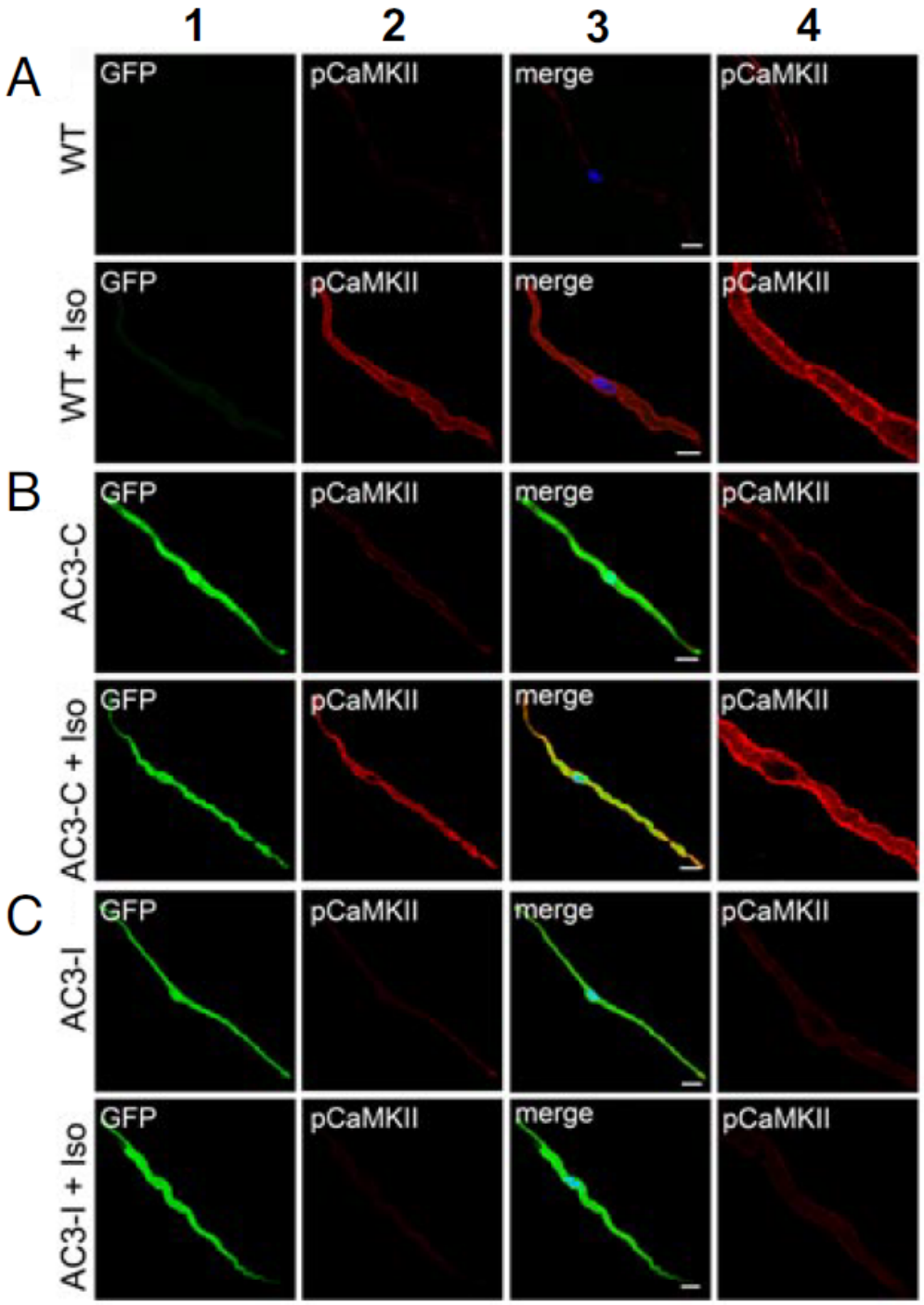
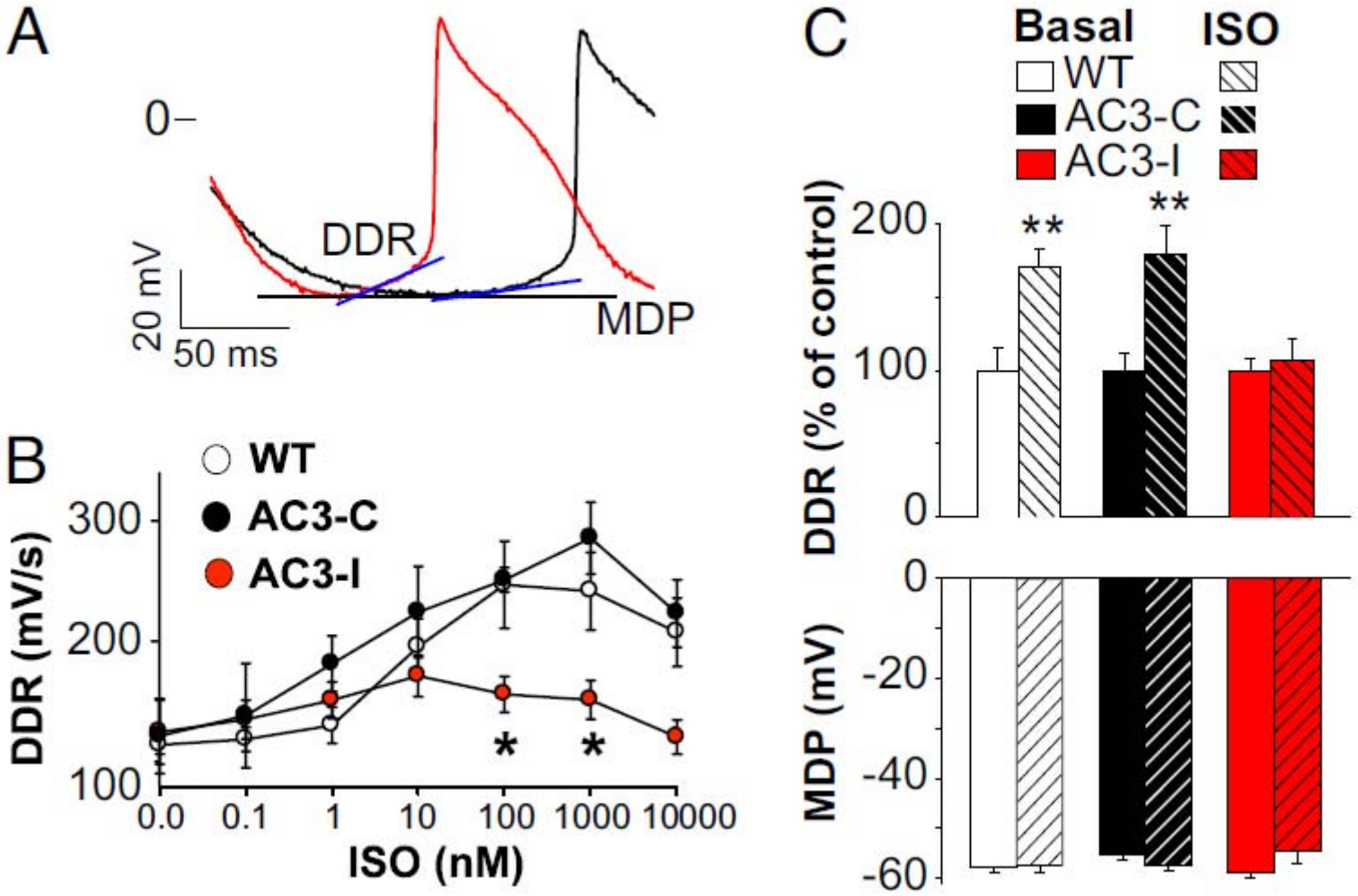
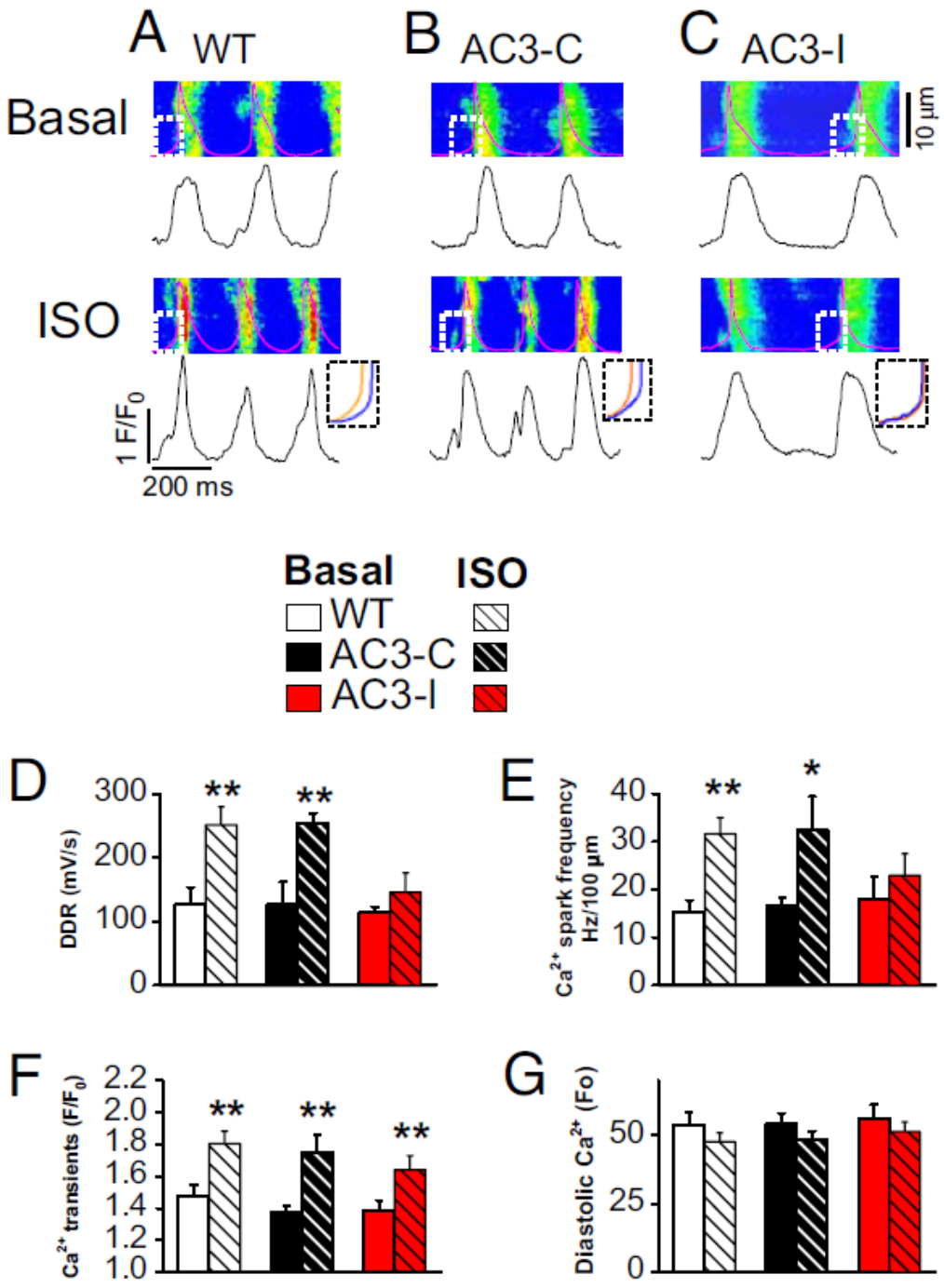

A
In vivo
(stress)
Heart Rate (beats/min)
800
600
400
200
0
***
B
In vivo
(rest)
Basal
ISO
WT
AC3-C
AC3-I
C
In vivo
(ISO)
*
D
Ex vivo
E
Baseline
ISO 1μM
WT
AC3-I
AC3-C
150 ms
F
650
550
450
350
0.0
0.1
1
10
100
1000
ISO (nM)

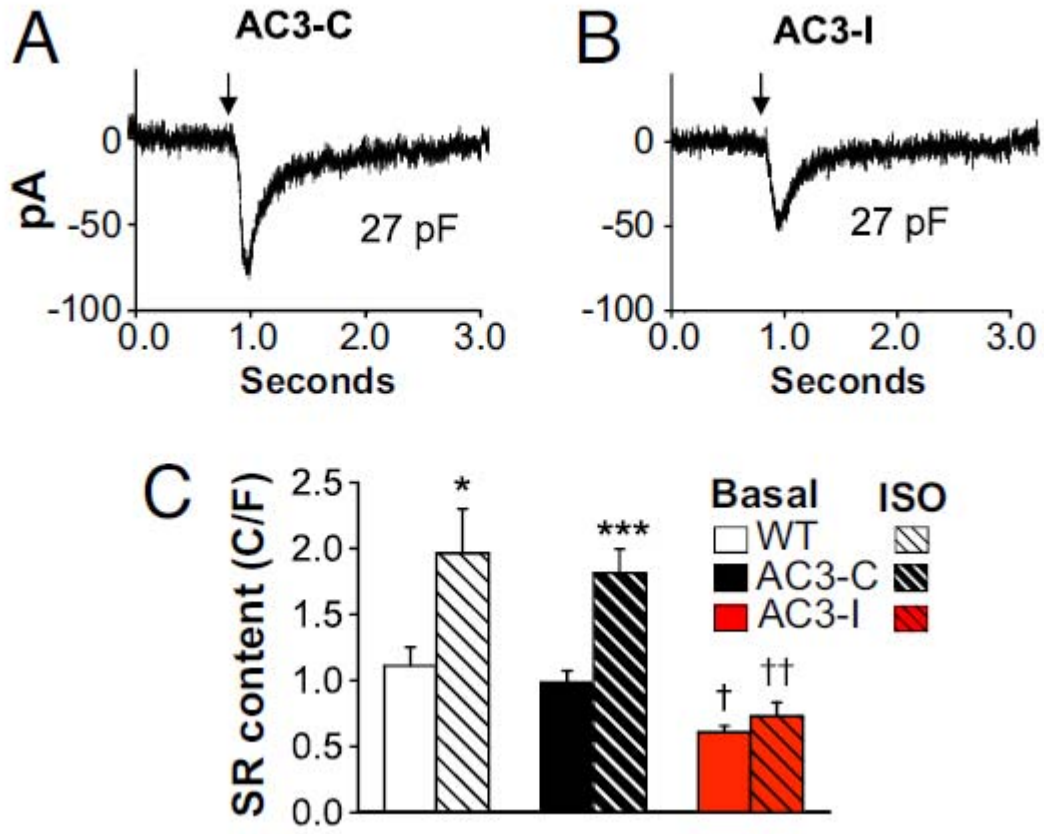

A
ISO
Baseline
0
20 mV
250 ms
B
WT
AC3-C
AC3-I
Beating rate (bpm)
450
400
350
300
250
**
**
*
0.0
0.1
1
10
100
1000
10000
ISO (nM)


1
2
3
4
A
WT
GFP
pCaMKII
merge
pCaMKII
WT + Iso
GFP
pCaMKII
merge
pCaMKII
B
AC3-C
GFP
pCaMKII
merge
pCaMKII
AC3-C + Iso
GFP
pCaMKII
merge
pCaMKII
C
AC3-I
GFP
pCaMKII
merge
pCaMKII
AC3-I + Iso
GFP
pCaMKII
merge
pCaMKII


A
0
DDR
MDP
20 mV
50 ms
B
WT
AC3-C
AC3-I
DDR (mV/s)
300
200
100
0.0
0.1
1
10
100
1000
10000
ISO (nM)
*
*
C
Basal
ISO
WT
AC3-C
AC3-I
DDR (% of control)
200
100
0
**
**
MDP (mV)
0
-20
-40
-60


A WT
B AC3-C
C AC3-I
Basal
ISO
10 µm
1 F/F0
200 ms
Basal
ISO
WT
AC3-C
AC3-I
D
DDR (mV/s)
300
200
100
0
**
**
E
Ca2+ spark frequency Hz/100 µm
40
30
20
10
0
**
*
F
Ca2+ transients (F/F0)
2.2
2.0
1.8
1.6
1.4
1.2
1.0
**
**
**
G
Diastolic Ca2+ (Fo)
50
25
0

SR Ca2 content is reduced in AC3-I SANcells



Ca^{2+}-Related Signaling and Protein Phosphorylation Abnormalities Play Central Roles in a New Experimental Model of Electrical Storm

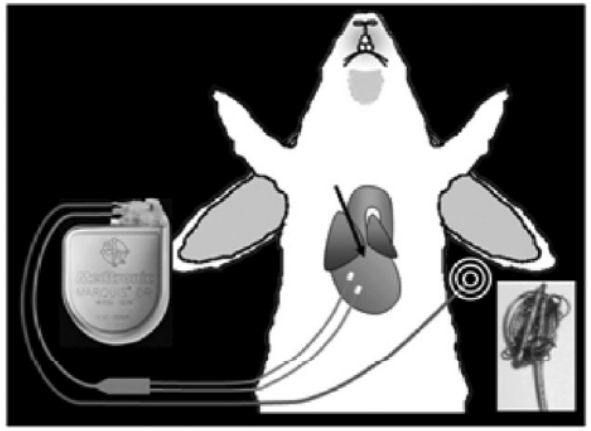
Yukiomi Tsuji, MD; Mayumi Hojo, BSc; Niels Voigt, MD; Ali El-Armouche, MD; Yasuya Inden, MD; Toyoaki Murohara, MD; Dobromir Dobrev, MD; Stanley Nattel, MD; Itsuo Kodama, MD; Kaichiro Kamiya, MD

Background—Electrical storm (ES), characterized by recurrent ventricular tachycardia/fibrillation, typically occurs in implantable cardioverter-defibrillator patients and adversely affects prognosis. However, the underlying molecular basis is poorly understood. In the present study, we report a new experimental model featuring repetitive episodes of implantable cardioverter-defibrillator firing for recurrent ventricular fibrillation (VF), in which we assessed involvement of Ca^{2+}-related protein alterations in ES.

Methods and Results—We studied 37 rabbits with complete atrioventricular block for ≈80 days, all with implantable cardioverter-defibrillator implantation. All rabbits showed long-QT and VF episodes. Fifty-three percent of rabbits developed ES (≥3 VF episodes per 24-hour period; 103±23 VF episodes per rabbit). Expression/phosphorylation of Ca^{2+}-handling proteins was assessed in left ventricular tissues from rabbits with the following: ES; VF episodes but not ES (non-ES); and controls. Left ventricular end-diastolic diameter increased comparably in ES and non-ES rabbits, but contractile dysfunction was significantly greater in ES than in non-ES rabbits. ES rabbits showed striking hyperphosphorylation of Ca^{2+}/calmodulin-dependent protein kinase II, prominent phospholamban dephosphorylation, and increased protein phosphatase 1 and 2A expression versus control and non-ES rabbits. Ryanodine receptors were similarly hyperphosphorylated at Ser2815 in ES and non-ES rabbits, but ryanodine receptor Ser2809 and L-type Ca^{2+} channel α-subunit hyperphosphorylation were significantly greater in ES versus non-ES rabbits. To examine direct effects of repeated VF/defibrillation, VF was induced 10 times in control rabbits. Repeated VF tissues showed autophosphorylated Ca^{2+}/calmodulin-dependent protein kinase II upregulation and phospholamban dephosphorylation like those of ES rabbit hearts. Continuous infusion of a calmodulin antagonist (W-7) to ES rabbits reduced Ca^{2+}/calmodulin-dependent protein kinase II hyperphosphorylation, suppressed ventricular tachycardia/fibrillation, and rescued left ventricular dysfunction.

Conclusions—ES causes Ca^{2+}/calmodulin-dependent protein kinase II activation and phospholamban dephosphorylation, which can explain the vicious cycle of arrhythmia promotion and mechanical dysfunction that characterizes ES. **(*Circulation*. 2011;123:2192-2203.)**

A

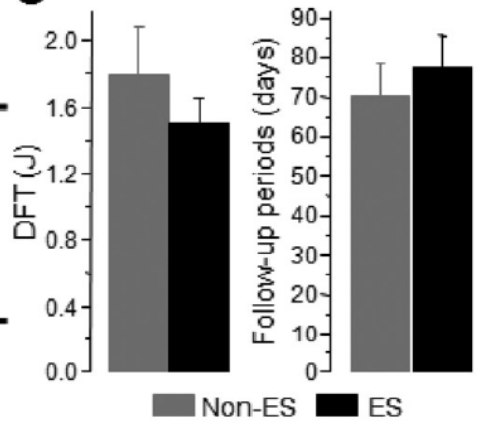


B

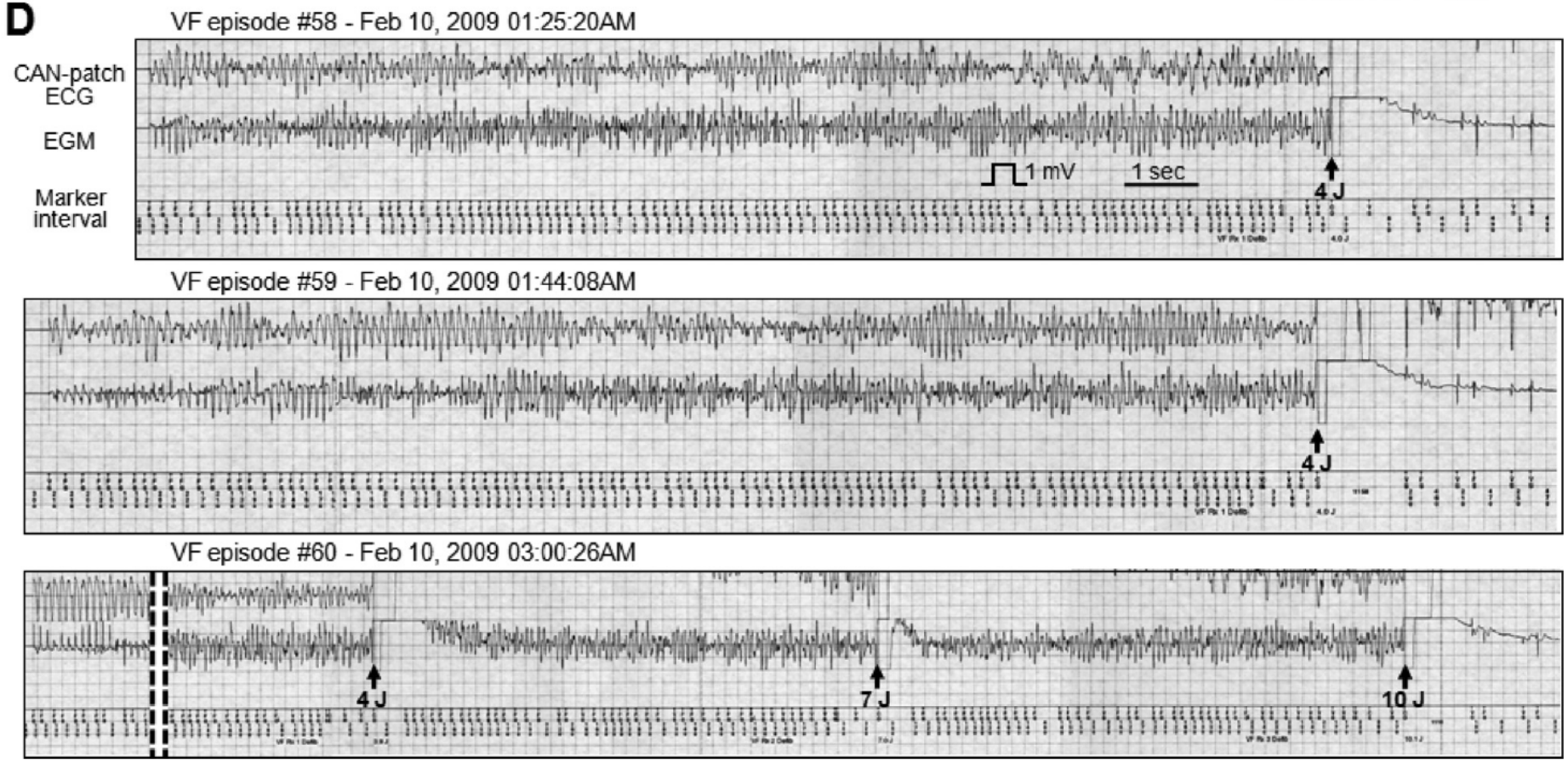
	Enable	Initial	Redetect	V Interval (Rate)
VF	On	120/160	30/40	<240 ms (>250 bpm)
VT	Off			

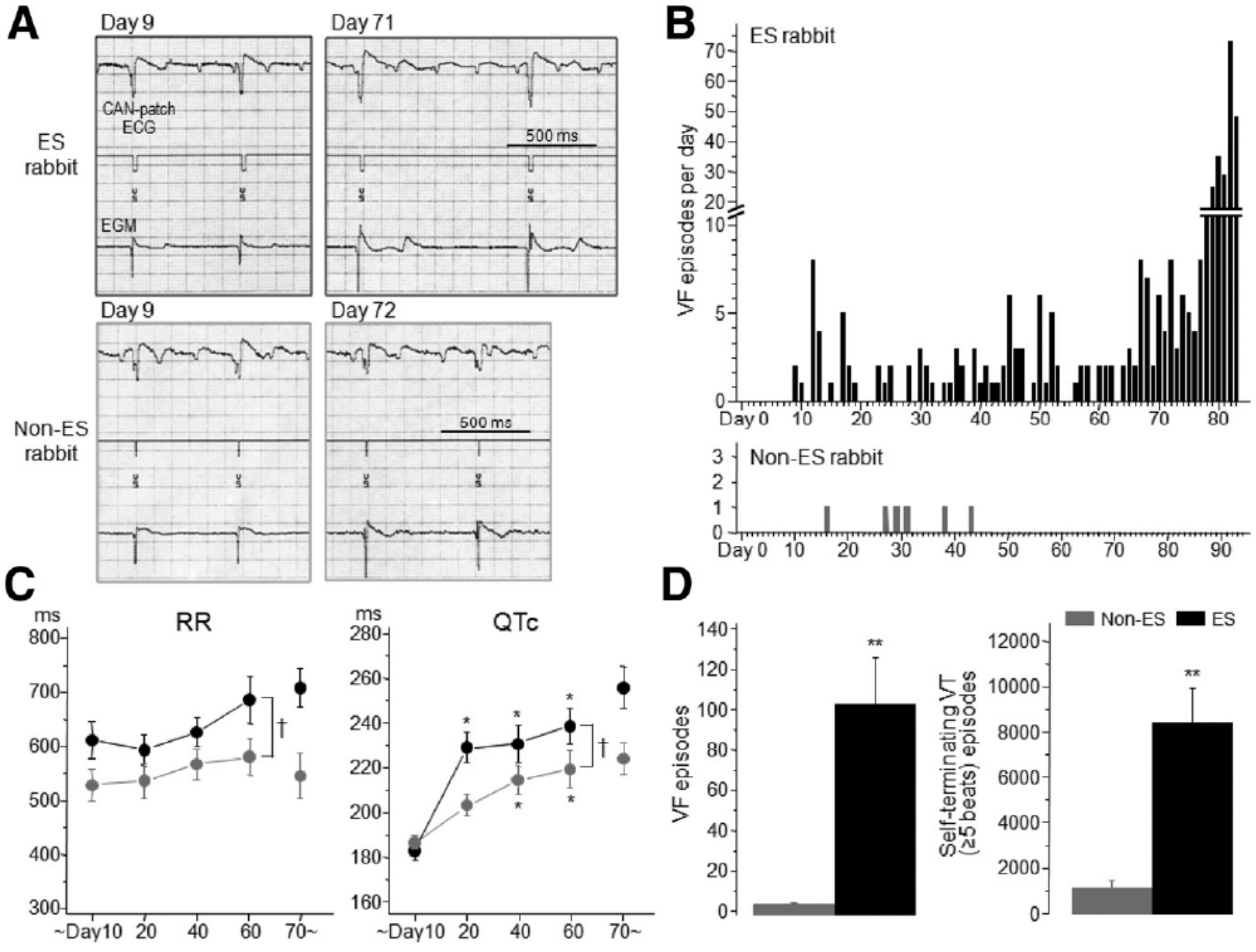
Therapy	Rx1	Rx2	Rx3	Rx4	Rx5	Rx6
VF	4J	7 J	10 J	12 J	14 J	16 J

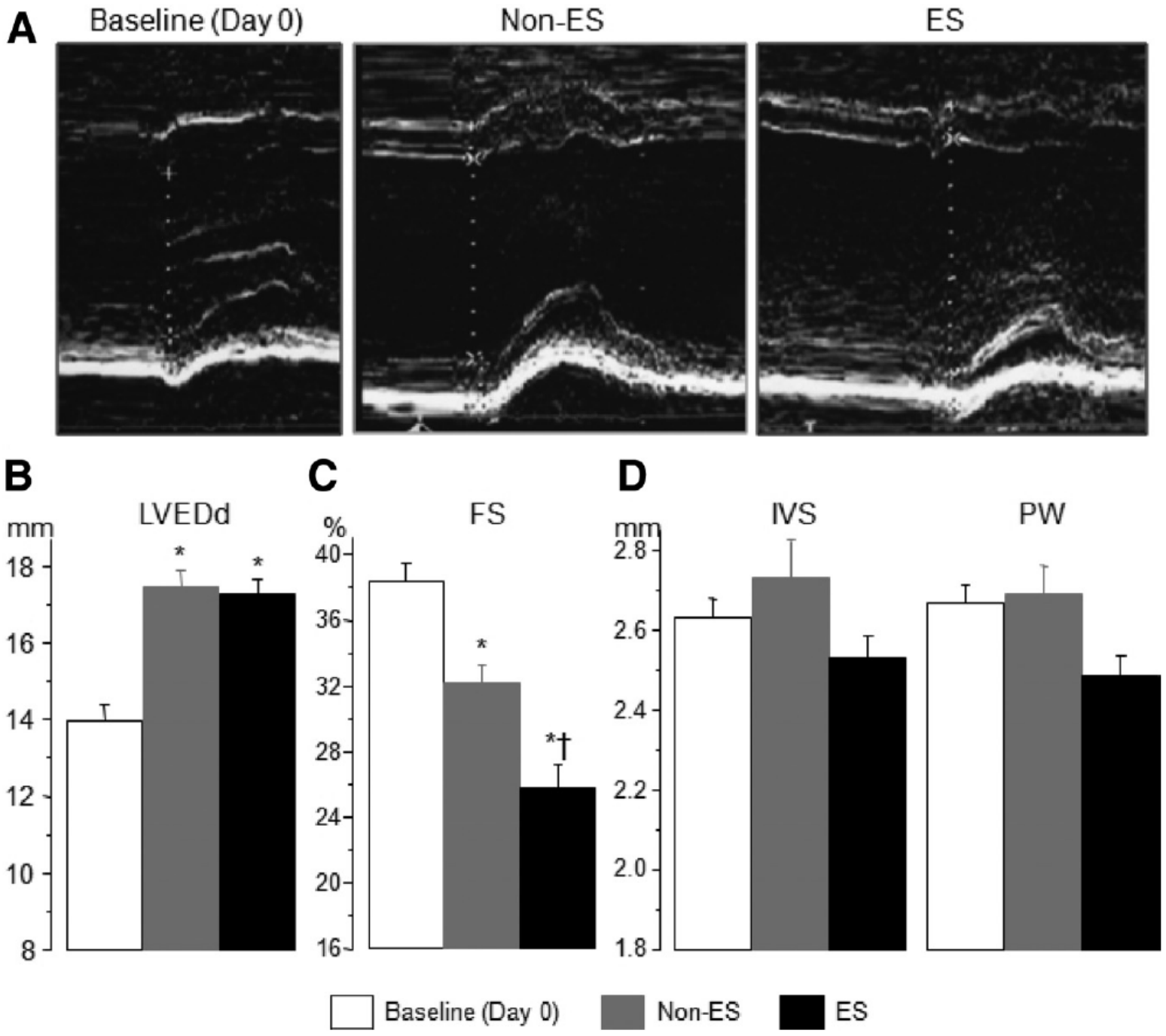
C

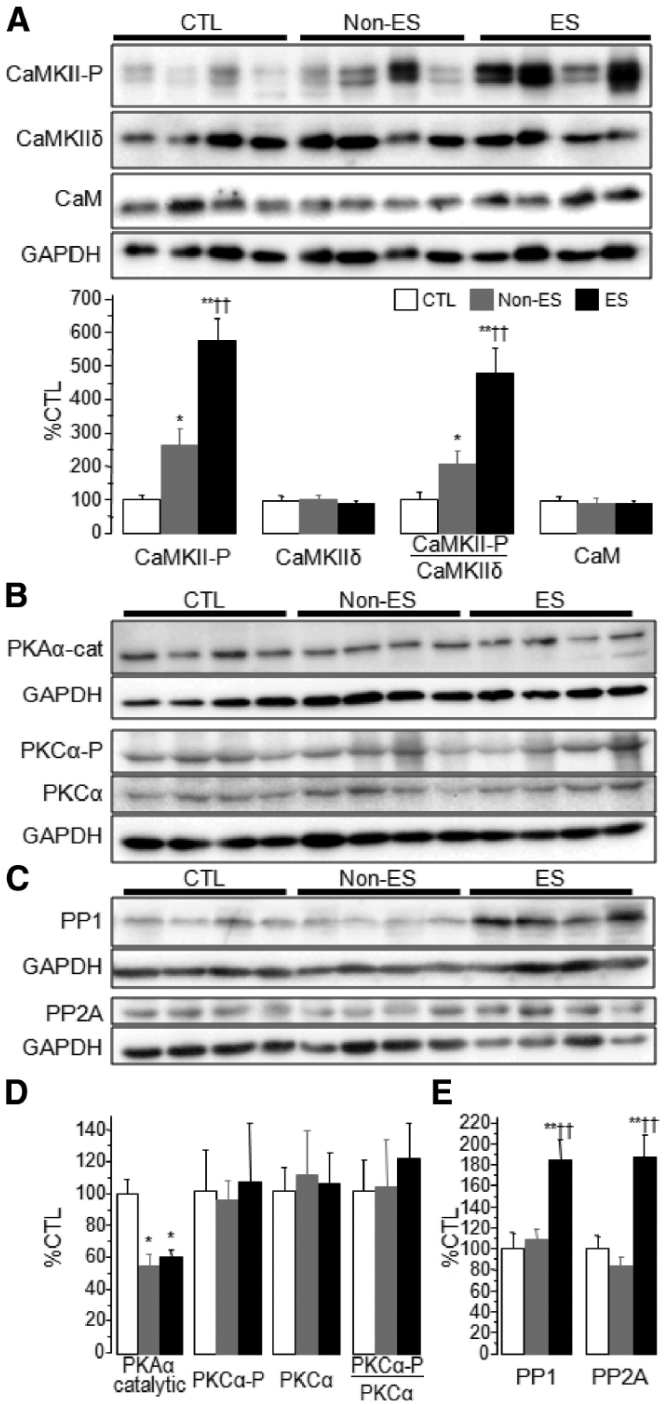


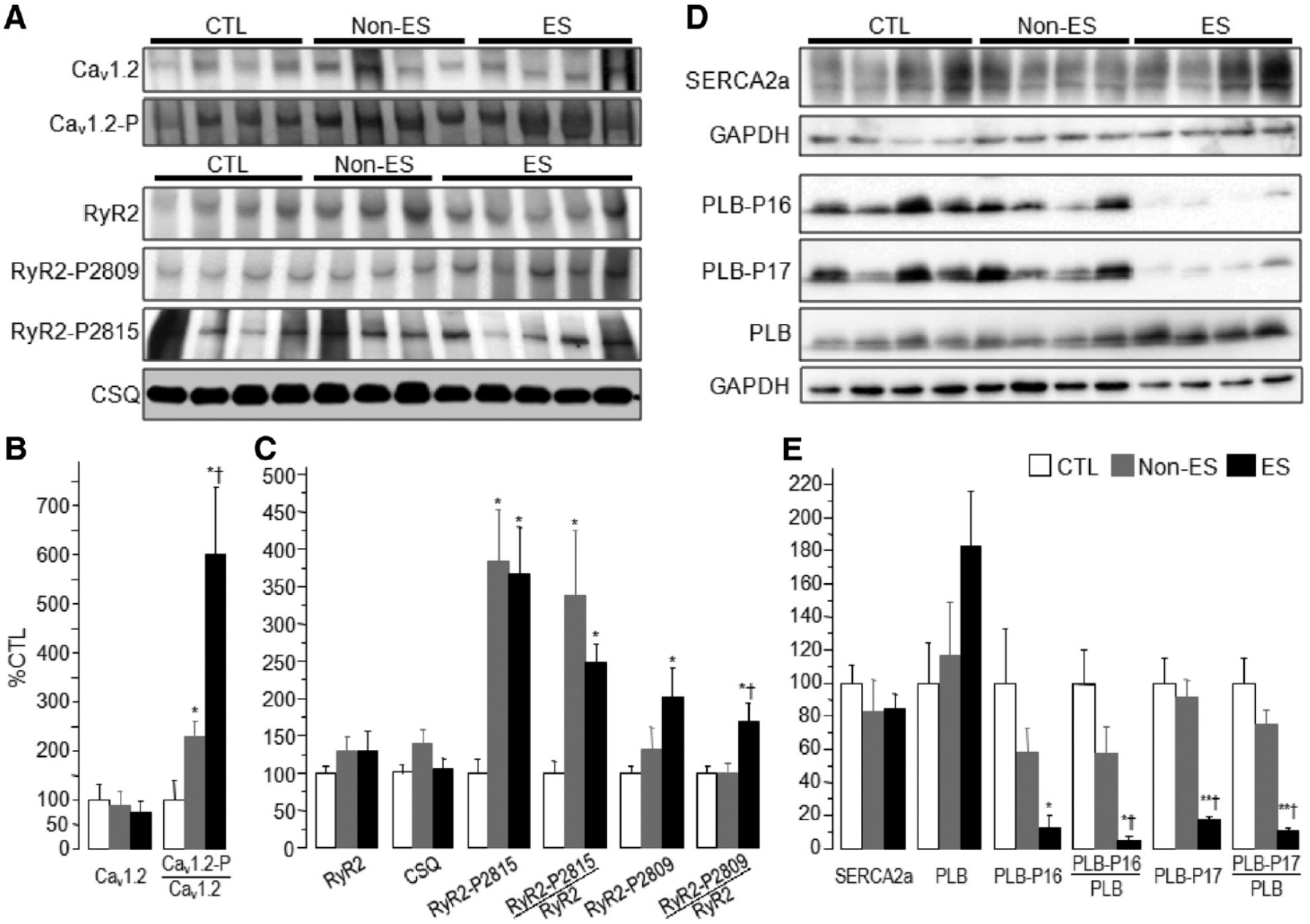
D

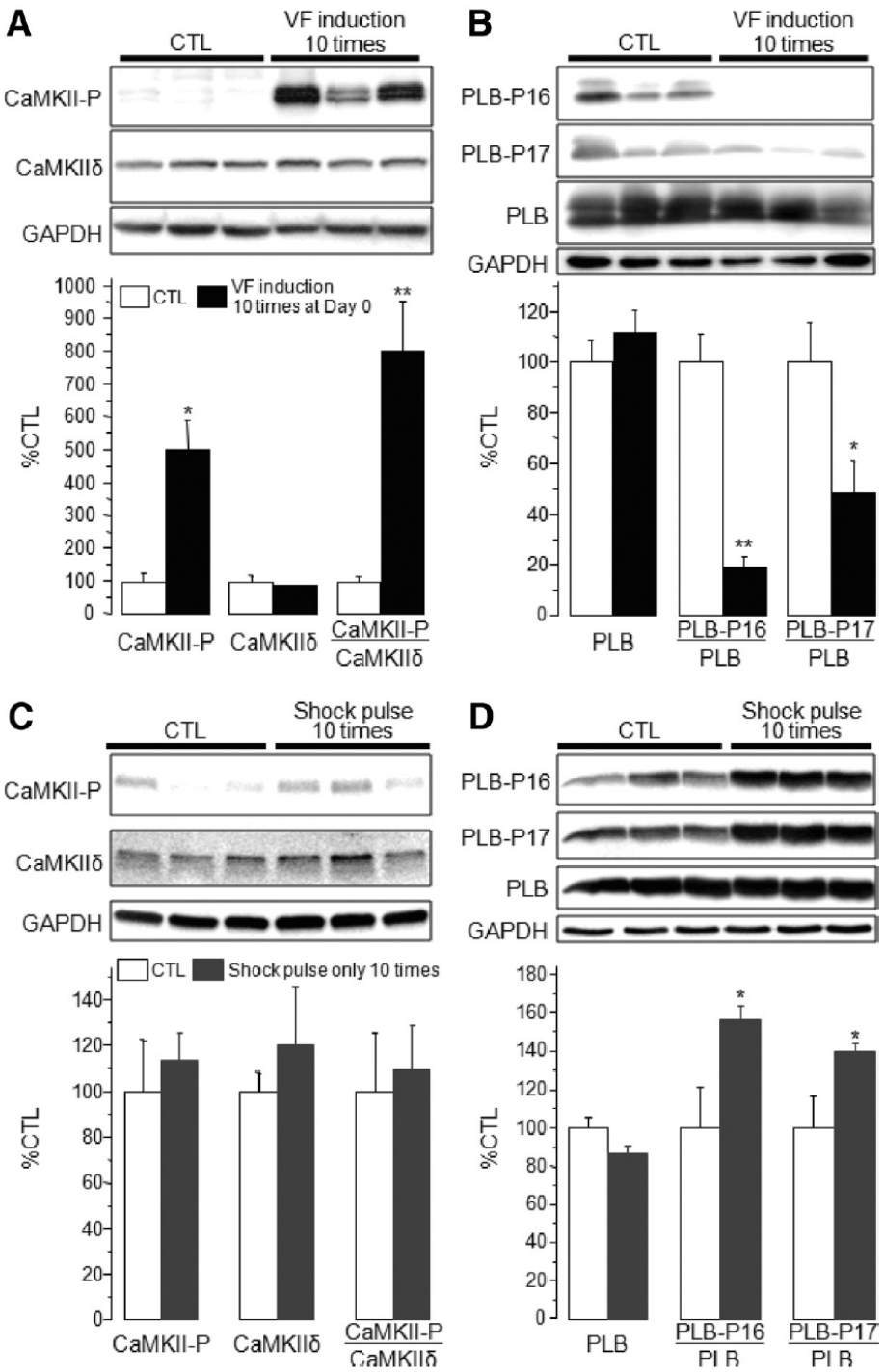


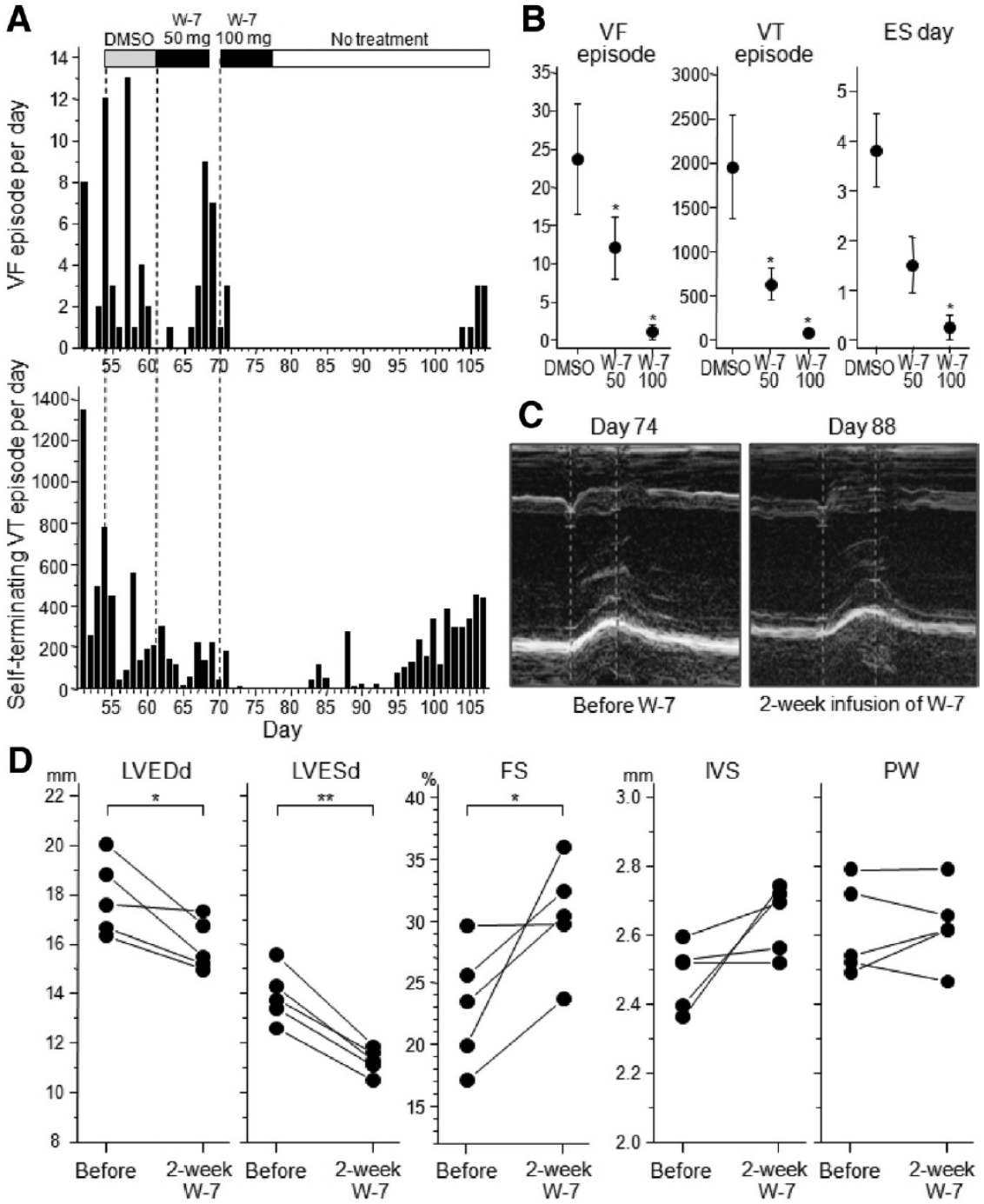

A
Day 9
Day 71
ES rabbit
CAN-patch ECG
EGM
500 ms
Day 9
Day 72
Non-ES rabbit
500 ms
B
ES rabbit
VF episodes per day
Day 0
Non-ES rabbit
C
RR
QTc
ms
~Day10
20
40
60
70~
D
VF episodes
Self-terminating VT (≥5 beats) episodes
Non-ES
ES

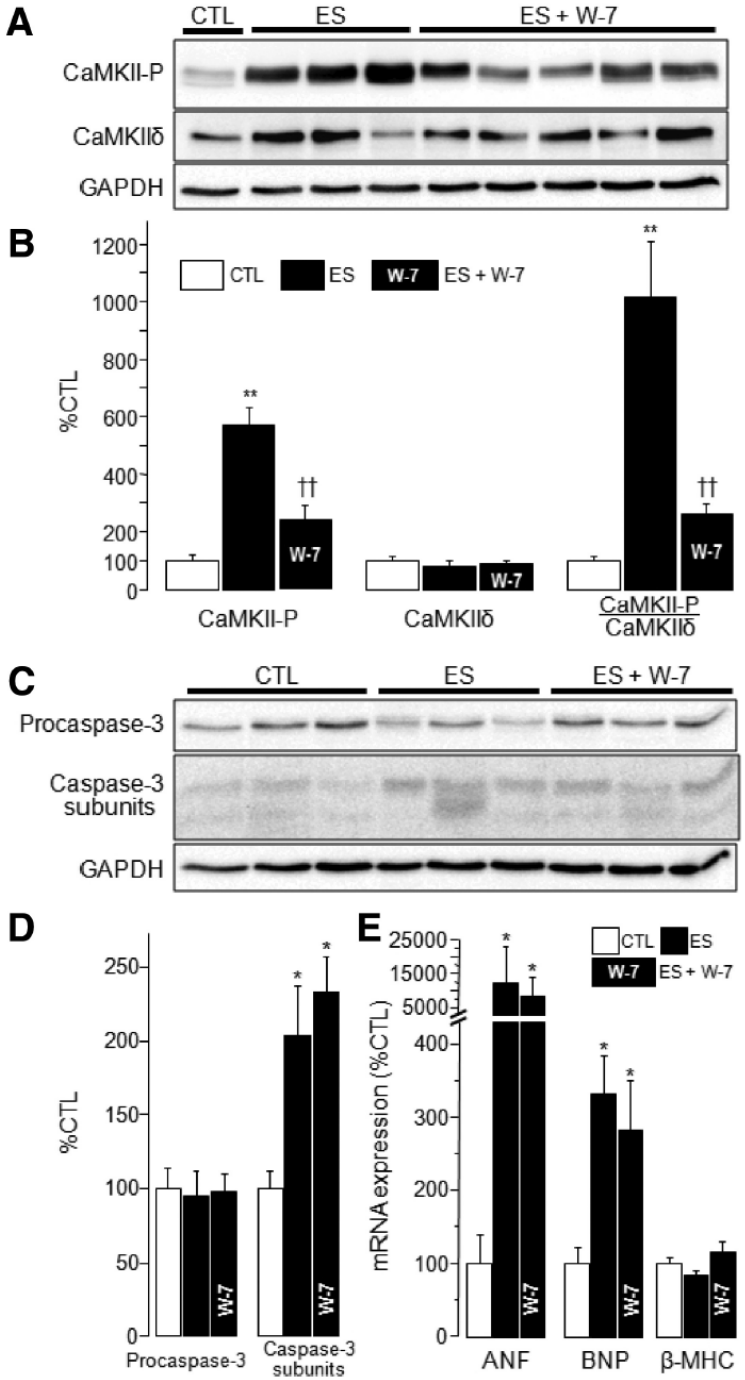

A
Baseline (Day 0)
Non-ES
ES
B
LVEDd
mm
18
16
14
12
10
8
C
FS
%
40
36
32
28
24
20
16
D
IVS
PW
mm
2.8
2.6
2.4
2.2
2.0
1.8
Baseline (Day 0)
Non-ES
ES

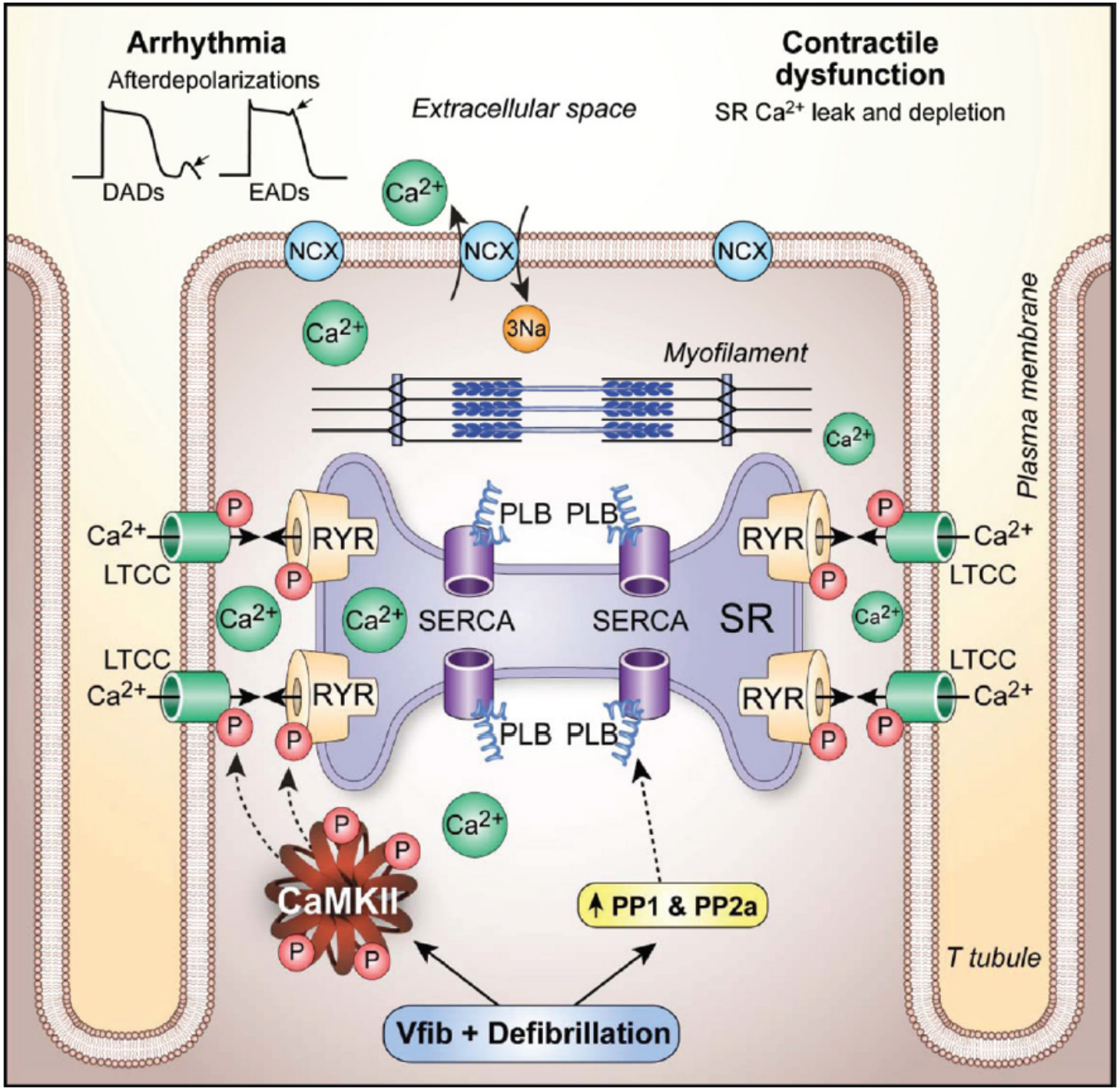

A
CTL
Non-ES
ES
CaMKII-P
CaMKIIδ
CaM
GAPDH
%CTL
CaMKII-P
CaMKIIδ
CaMKII-P / CaMKIIδ
CaM
B
PKAα-cat
GAPDH
PKCα-P
PKCα
C
PP1
PP2A
D
PKAα catalytic
PKCα-P
PKCα
PKCα-P / PKCα
E
PP1
PP2A


A
CTL
Non-ES
ES
Cav1.2
Cav1.2-P
CTL
Non-ES
ES
RyR2
RyR2-P2809
RyR2-P2815
CSQ
D
CTL
Non-ES
ES
SERCA2a
GAPDH
PLB-P16
PLB-P17
PLB
GAPDH
B
%CTL
700
600
500
400
300
200
100
50
0
*†
*
Cav1.2
Cav1.2-P / Cav1.2
C
500
450
400
350
300
250
200
150
100
50
0
*
*
*
*
*
*†
RyR2
CSQ
RyR2-P2815
RyR2-P2815 / RyR2
RyR2-P2809
RyR2-P2809 / RyR2
E
CTL
Non-ES
ES
220
200
180
160
140
120
100
80
60
40
20
0
*
*†
**†
**†
SERCA2a
PLB
PLB-P16
PLB-P16 / PLB
PLB-P17
PLB-P17 / PLB


A
CTL
VF induction
10 times
CaMKII-P
CaMKIIδ
GAPDH
CTL
VF induction
10 times at Day 0
%CTL
CaMKII-P
CaMKIIδ
CaMKII-P
CaMKIIδ
B
CTL
VF induction
10 times
PLB-P16
PLB-P17
PLB
GAPDH
%CTL
PLB
PLB-P16
PLB
PLB-P17
PLB
C
CTL
Shock pulse
10 times
CaMKII-P
CaMKIIδ
GAPDH
CTL
Shock pulse only 10 times
%CTL
CaMKII-P
CaMKIIδ
CaMKII-P
CaMKIIδ
D
CTL
Shock pulse
10 times
PLB-P16
PLB-P17
PLB
GAPDH
%CTL
PLB
PLB-P16
PLB
PLB-P17
PLB


A
DMSO
W-7 50 mg
W-7 100 mg
No treatment
VF episode per day
Self-terminating VT episode per day
Day
B
VF episode
VT episode
ES day
DMSO
W-7 50
W-7 100
C
Day 74
Day 88
Before W-7
2-week infusion of W-7
D
LVEDd
LVESd
FS
IVS
PW
mm
%
Before
2-week W-7


A
CTL
ES
ES + W-7
CaMKII-P
CaMKIIδ
GAPDH
B
CTL
ES
W-7
ES + W-7
%CTL
1200
1000
800
600
400
200
100
0
**
††
CaMKII-P
CaMKIIδ
CaMKII-P
CaMKIIδ
C
CTL
ES
ES + W-7
Procaspase-3
Caspase-3 subunits
GAPDH
D
%CTL
250
200
150
100
50
0
Procaspase-3
Caspase-3 subunits
E
CTL
ES
W-7
ES + W-7
mRNA expression (%CTL)
25000
15000
5000
400
300
200
100
0
ANF
BNP
β-MHC


Arrhythmia
Afterdepolarizations
DADs
EADs
Extracellular space
Contractile dysfunction
SR Ca2+ leak and depletion
Ca2+
NCX
NCX
NCX
3Na
Myofilament
Plasma membrane
Ca2+
P
RYR
PLB
PLB
SERCA
SERCA
SR
LTCC
CaMKII
PP1 & PP2a
T tubule
Vfib + Defibrillation

Fatty Acid Synthase Modulates Homeostatic Responses to Myocardial Stress*[S]

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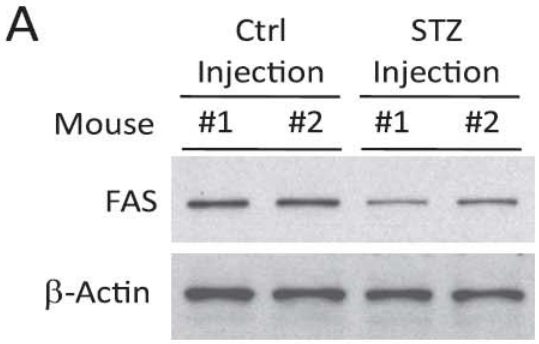
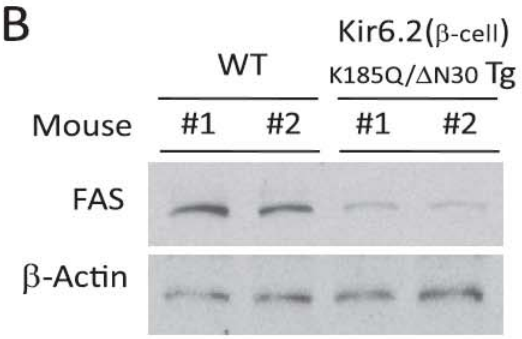
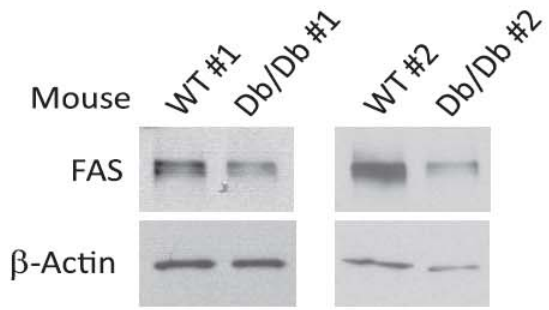
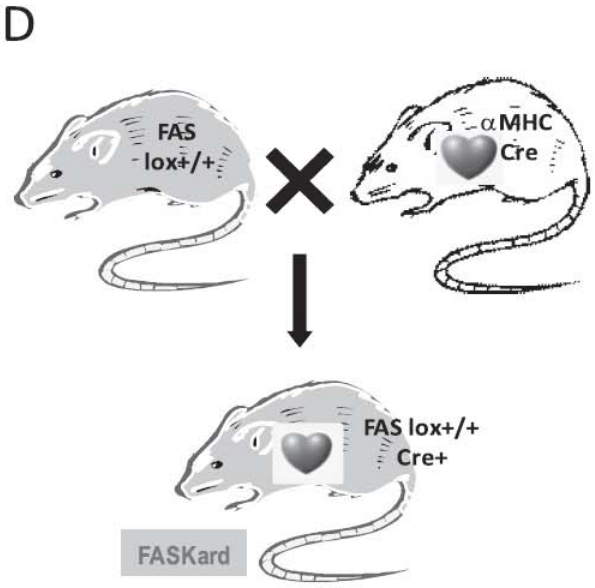
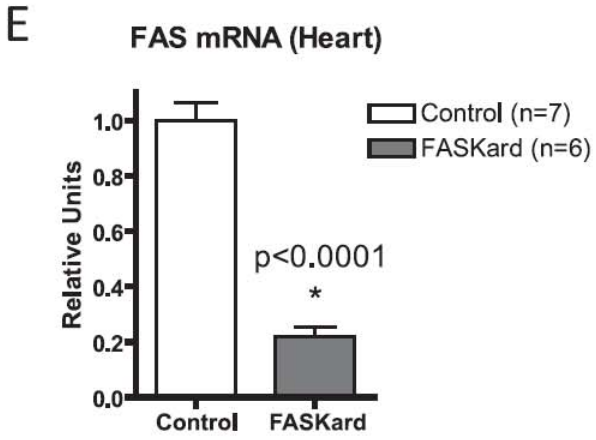
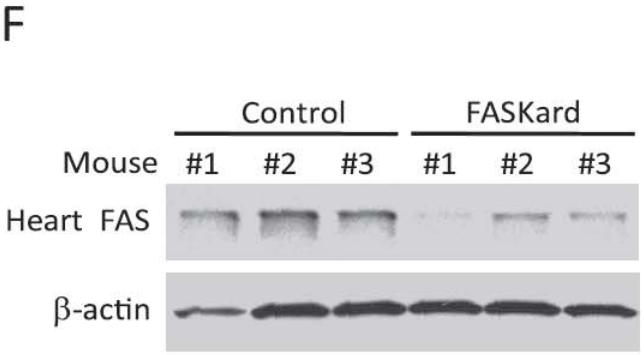

A
Ctrl Injection
STZ Injection
Mouse
#1
#2
#1
#2
FAS
β-Actin

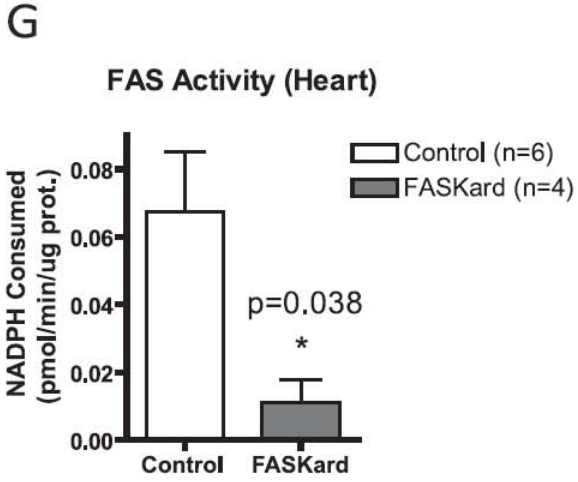

B
WT
Kir6.2(β-cell) K185Q/ΔN30 Tg
Mouse
#1
#2
#1
#2
FAS
β-Actin

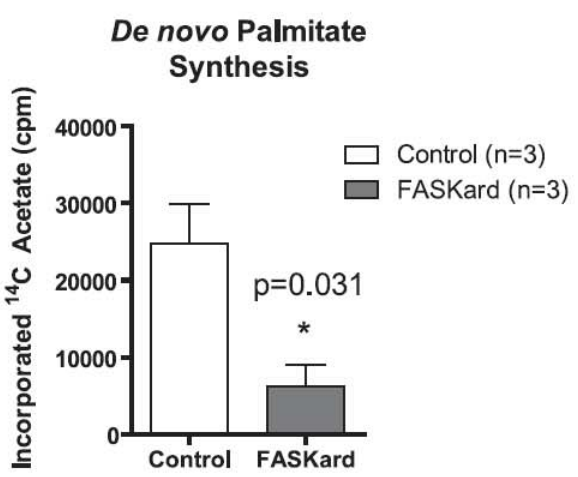

C
Mouse
WT #1
Db/Db #1
WT #2
Db/Db #2
FAS
β-Actin

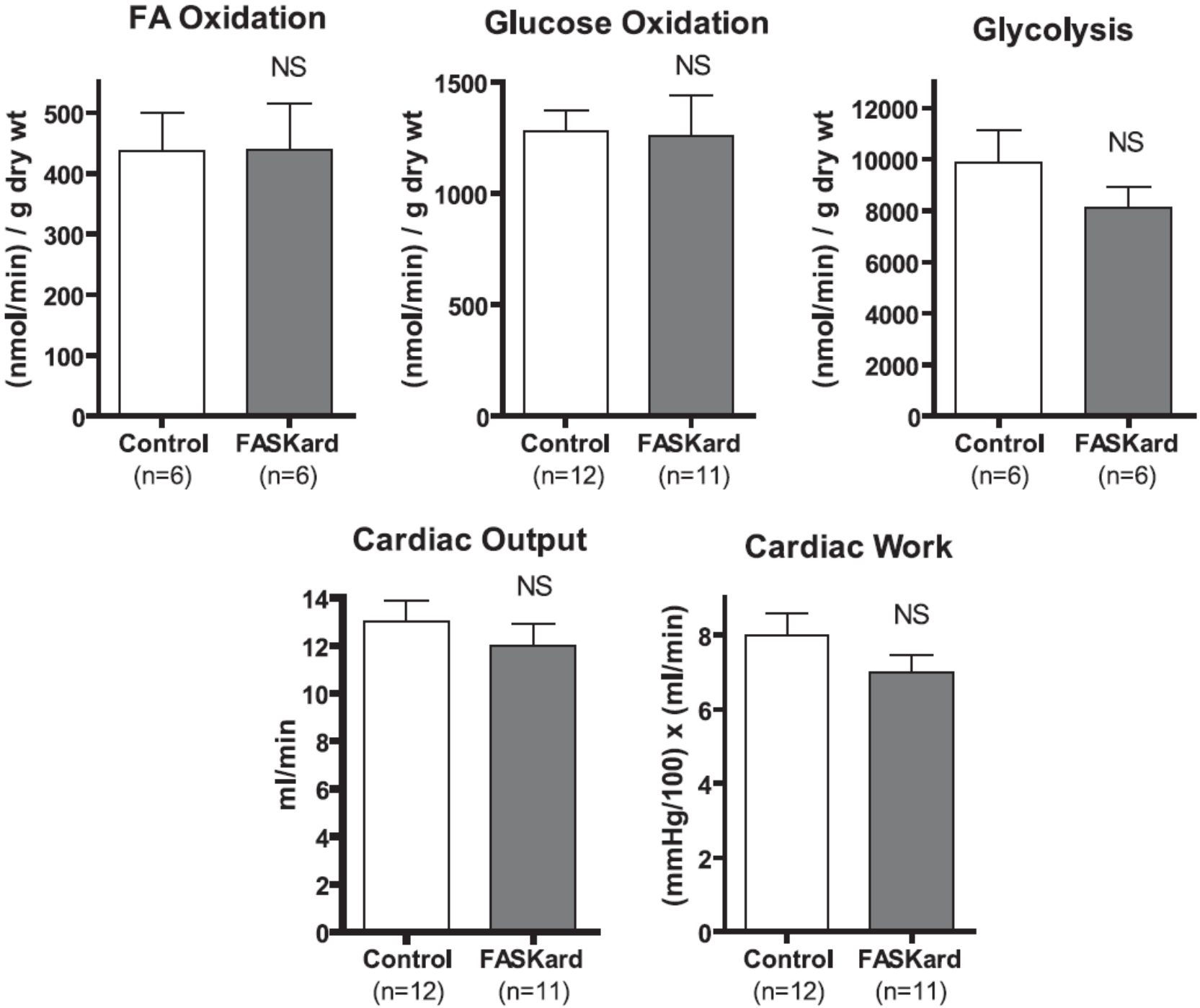

D
FAS lox+/+
αMHC Cre
FAS lox+/+ Cre+
FASKard

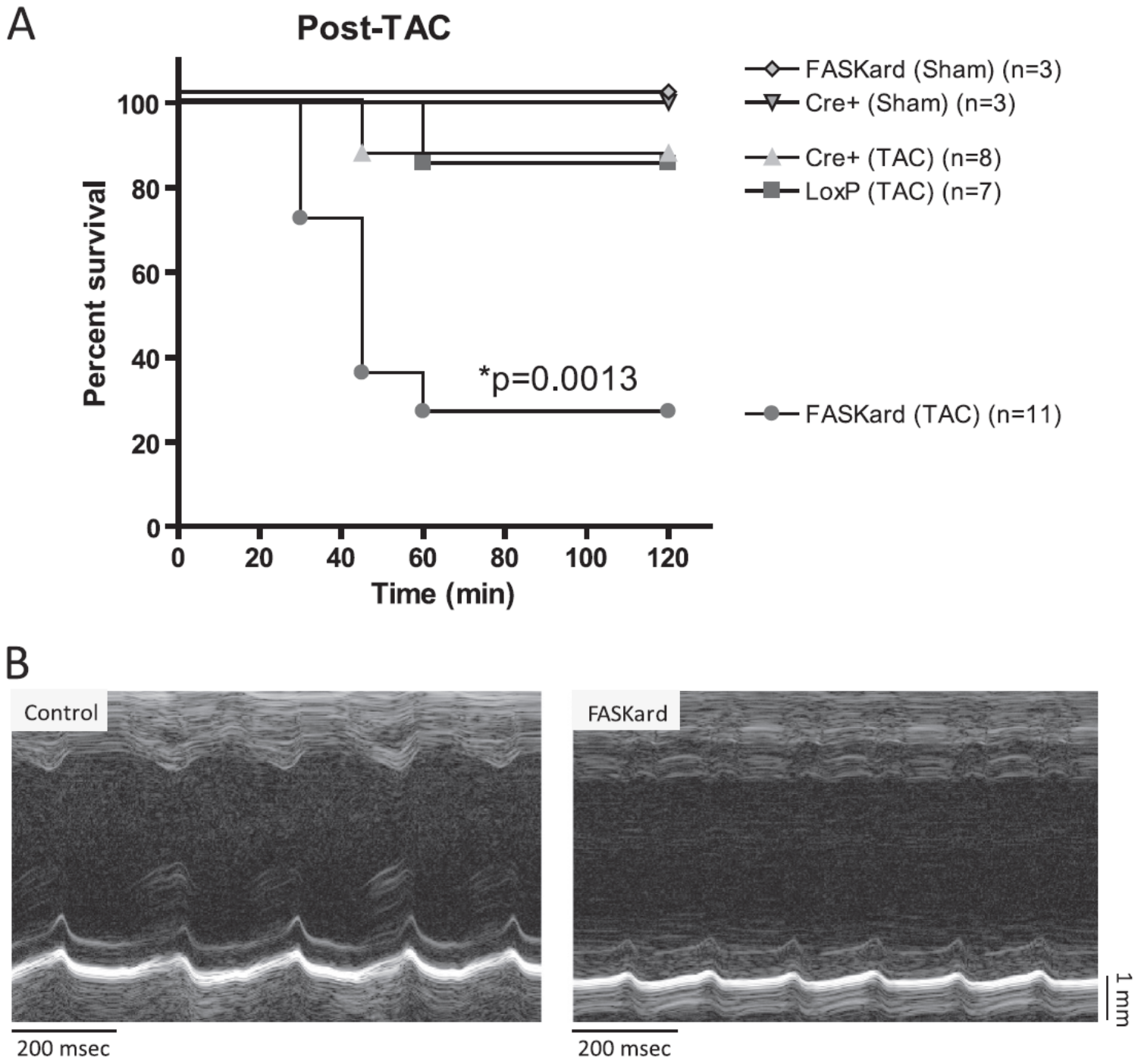

E
FAS mRNA (Heart)
Relative Units
1.0
0.8
0.6
0.4
0.2
0.0
Control (n=7)
FASKard (n=6)
p<0.0001
*
Control
FASKard

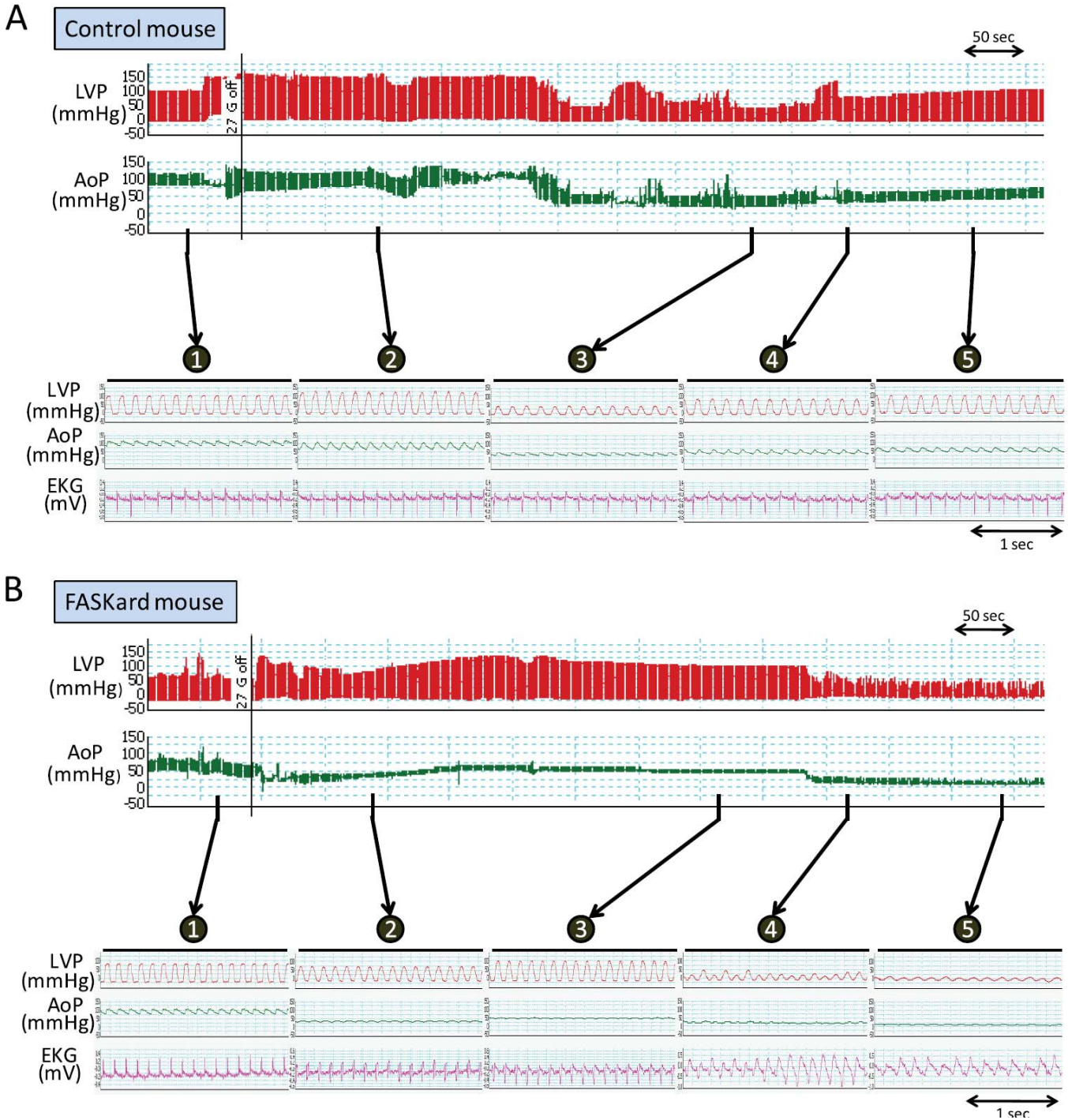

F
Control
FASKard
Mouse
#1
#2
#3
#1
#2
#3
Heart FAS
β-actin

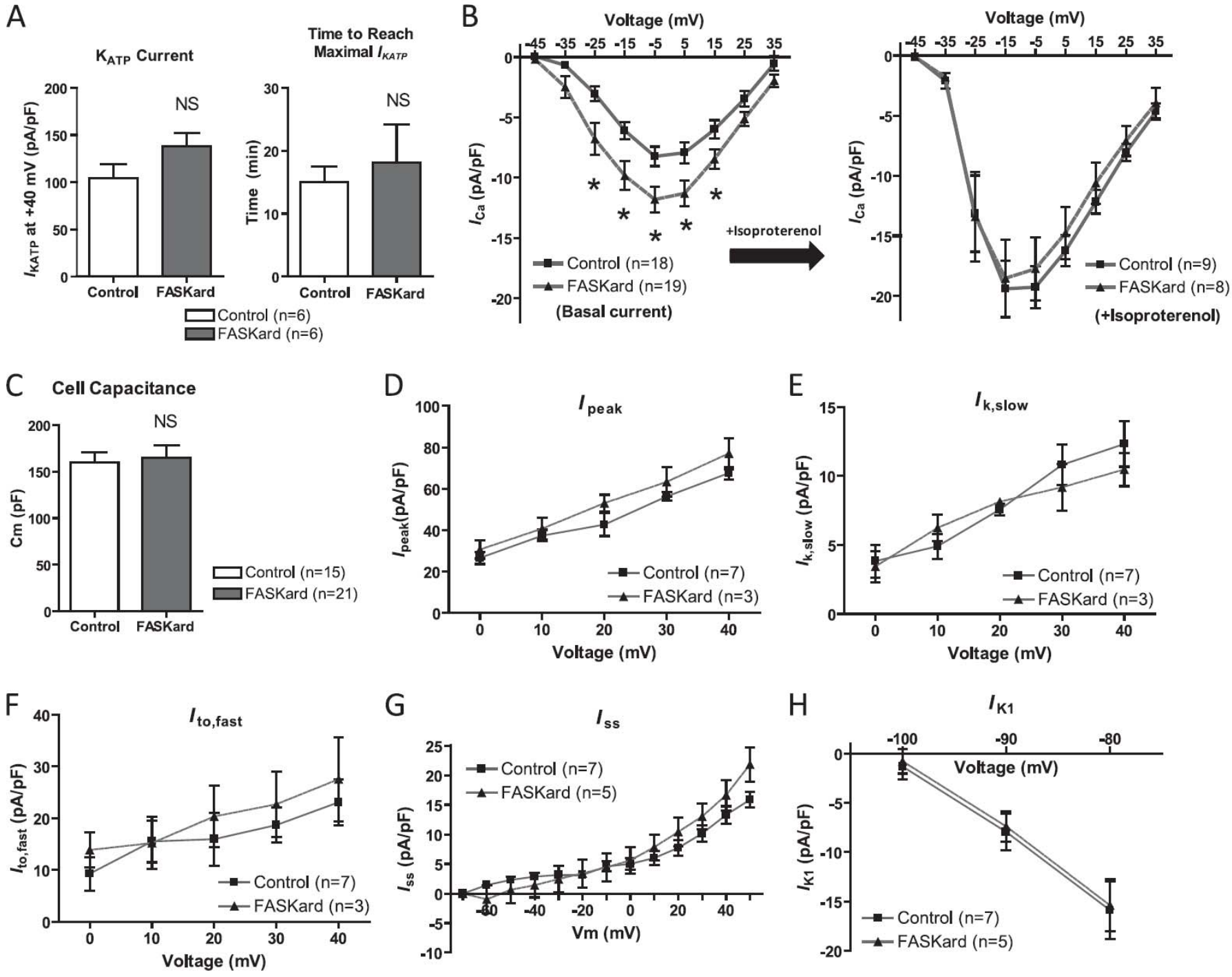

G
FAS Activity (Heart)
NADPH Consumed (pmol/min/ug prot.)
0.08
0.06
0.04
0.02
0.00
Control (n=6)
FASKard (n=4)
p=0.038
*
Control
FASKard

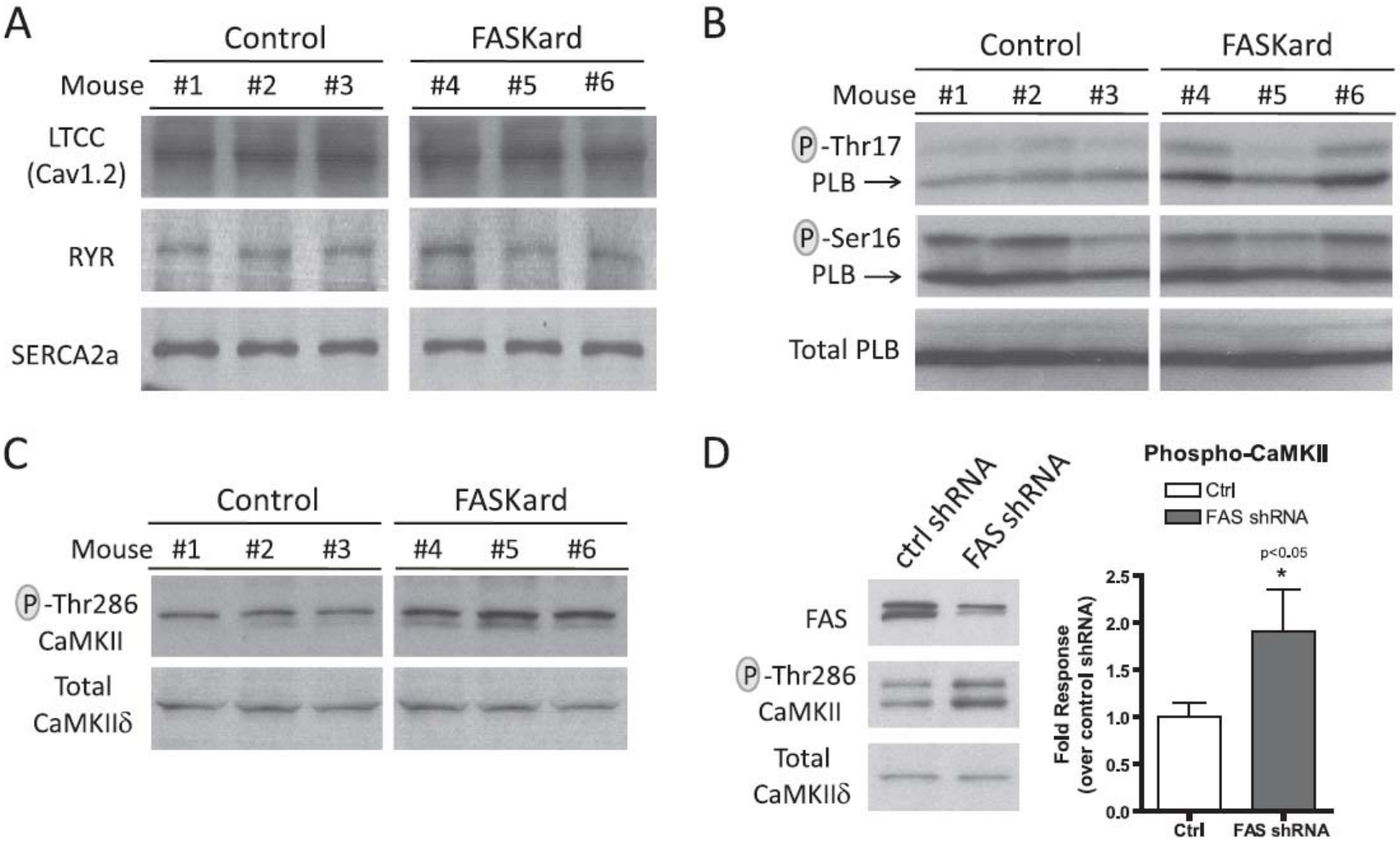

H
De novo Palmitate Synthesis
Incorporated 14C Acetate (cpm)
40000
30000
20000
10000
0
Control (n=3)
FASKard (n=3)
p=0.031
*
Control
FASKard

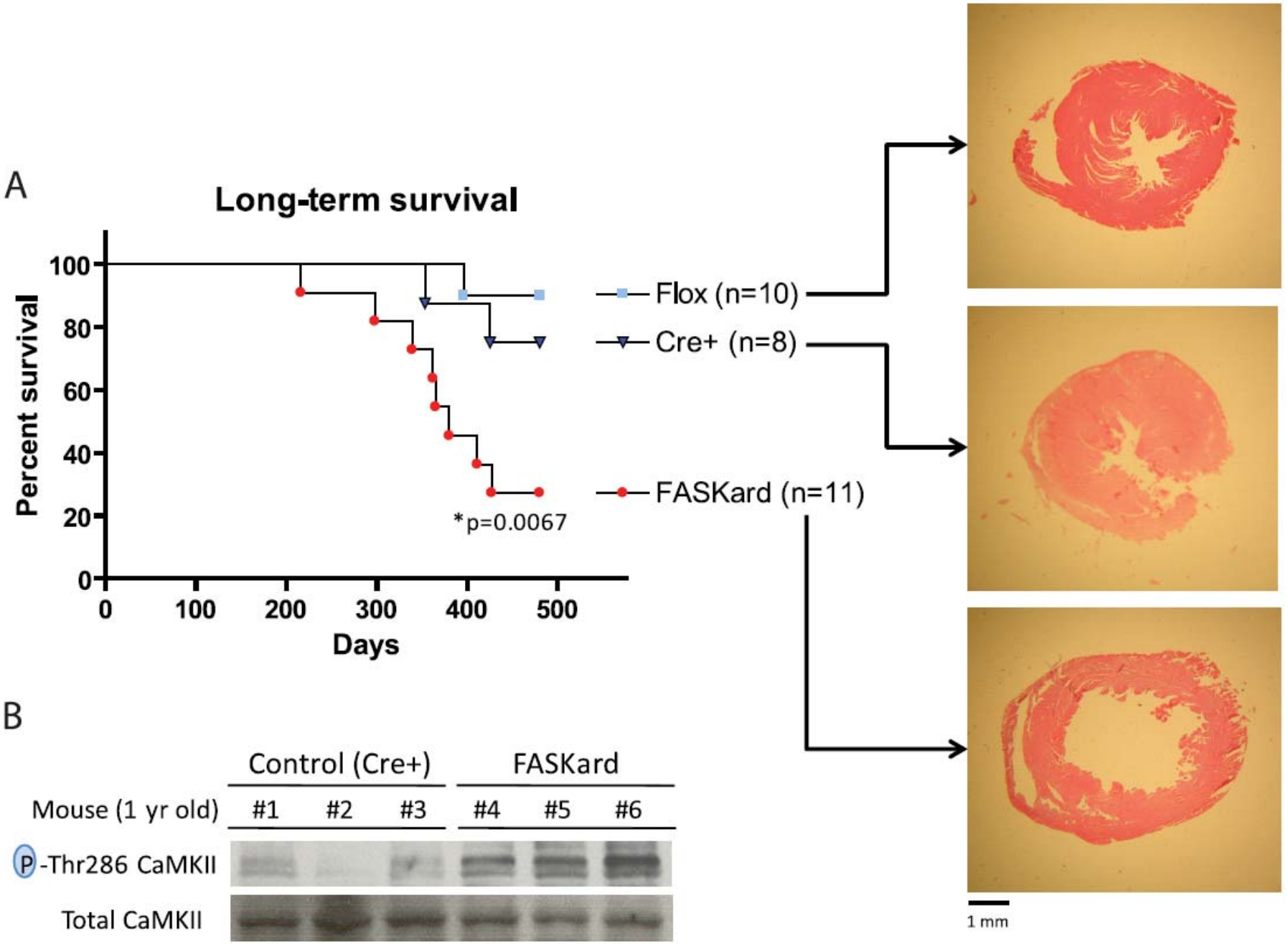

FA Oxidation
NS
(nmol/min) / g dry wt
500
400
300
200
100
0
Control
(n=6)
FASKard
(n=6)
Glucose Oxidation
NS
(nmol/min) / g dry wt
1500
1000
500
0
Control
(n=12)
FASKard
(n=11)
Glycolysis
NS
(nmol/min) / g dry wt
12000
10000
8000
6000
4000
2000
0
Control
(n=6)
FASKard
(n=6)
Cardiac Output
NS
ml/min
14
12
10
8
6
4
2
0
Control
(n=12)
FASKard
(n=11)
Cardiac Work
NS
(mmHg/100) x (ml/min)
8
6
4
2
0
Control
(n=12)
FASKard
(n=11)

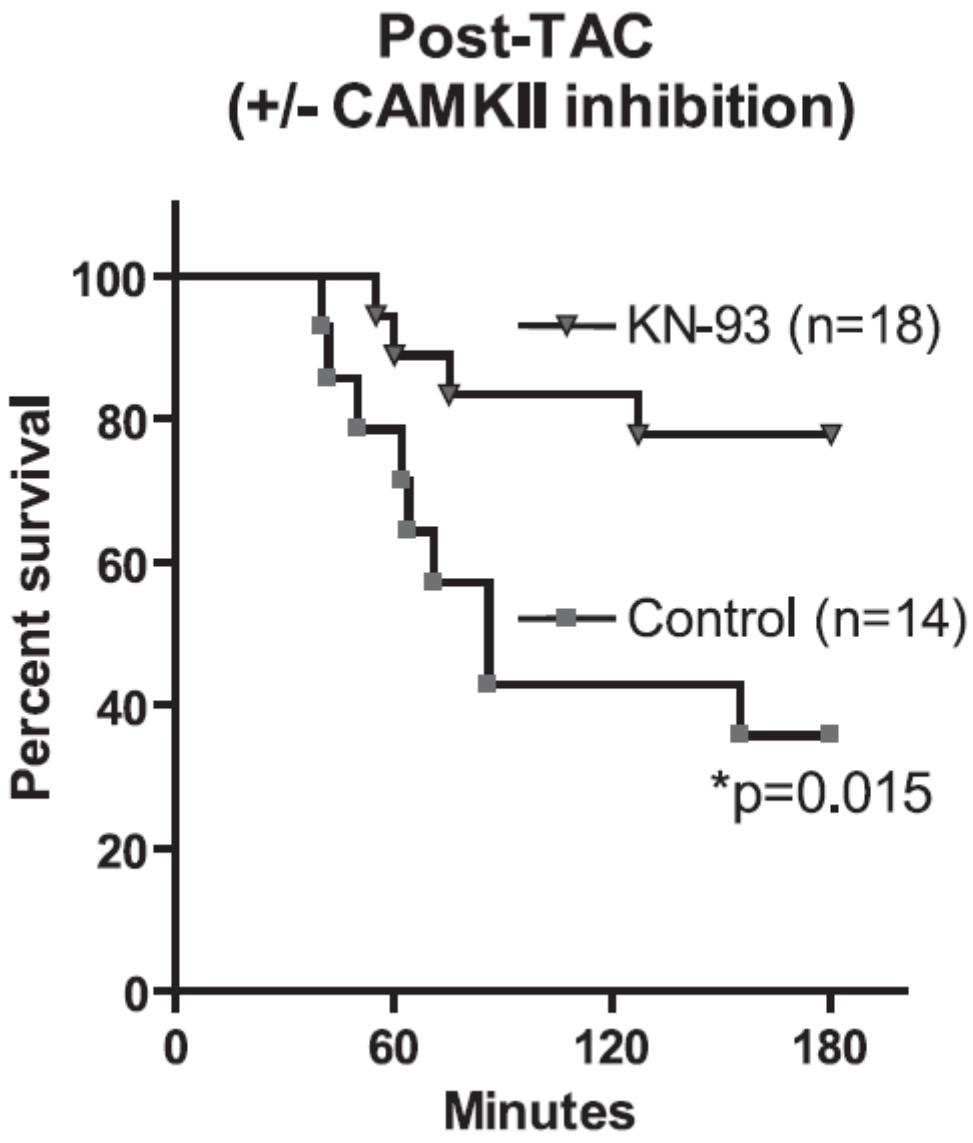

A
Post-TAC
Percent survival
100
80
60
40
20
0
0
20
40
60
80
100
120
Time (min)
*p=0.0013
FASKard (Sham) (n=3)
Cre+ (Sham) (n=3)
Cre+ (TAC) (n=8)
LoxP (TAC) (n=7)
FASKard (TAC) (n=11)
B
Control
FASKard
200 msec
200 msec
1 mm

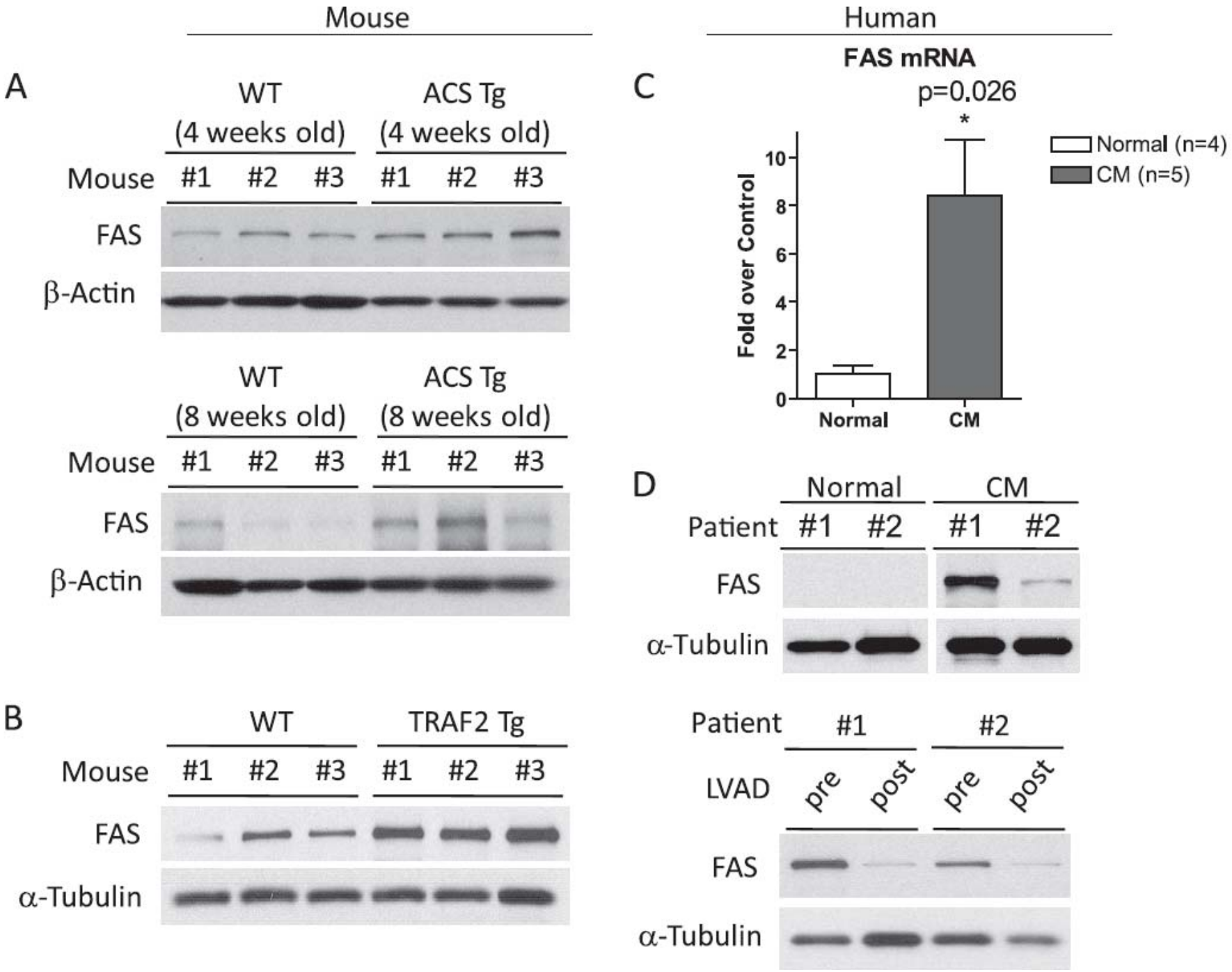

A
Control mouse
50 sec
LVP
(mmHg)
150
100
50
0
-50
2.7 G off
AoP
(mmHg)
150
100
50
0
-50
1
2
3
4
5
LVP
(mmHg)
AoP
(mmHg)
EKG
(mV)
1 sec
B
FASKard mouse
50 sec
LVP
(mmHg)
150
100
50
0
-50
2.7 G off
AoP
(mmHg)
150
100
50
0
-50
1
2
3
4
5
LVP
(mmHg)
AoP
(mmHg)
EKG
(mV)
1 sec

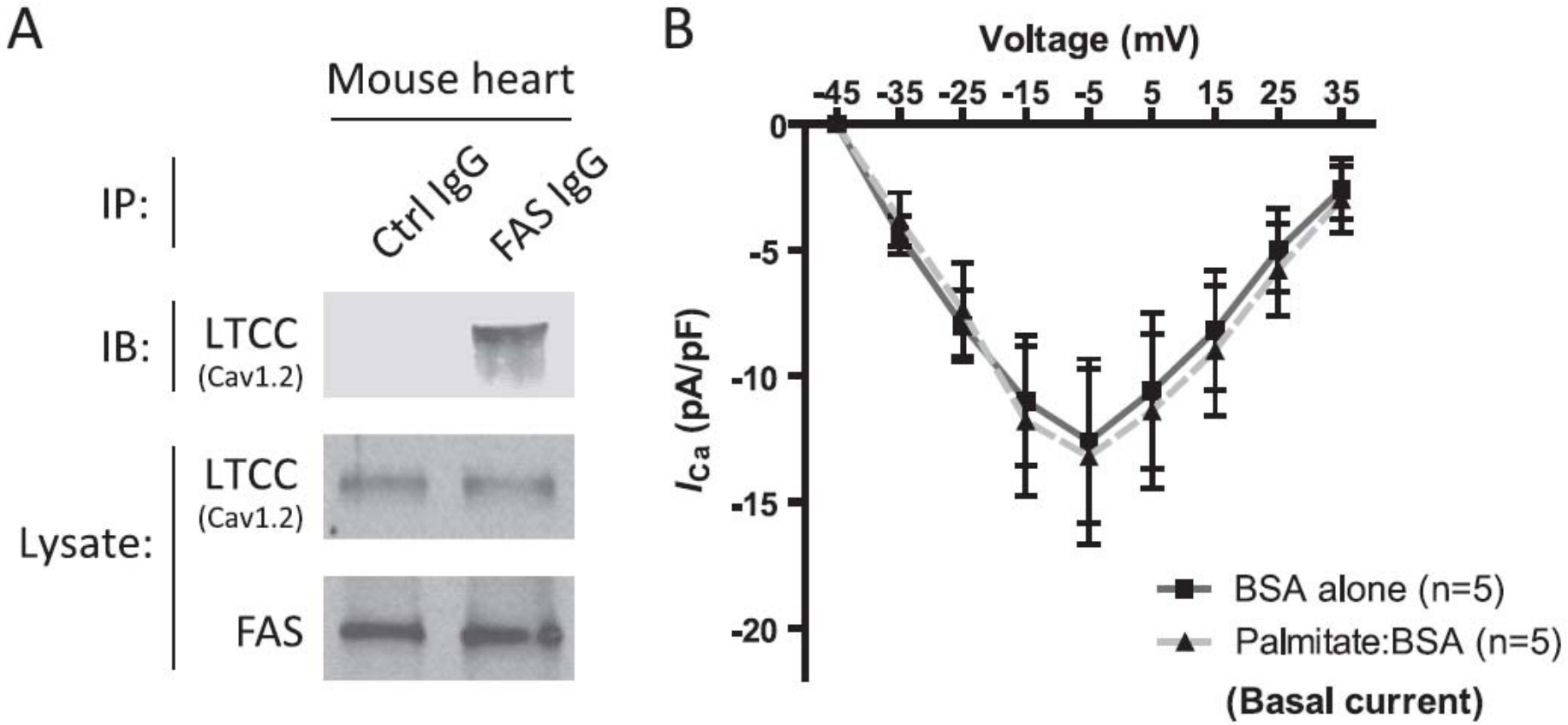

A
K_ATP Current
I_KATP at +40 mV (pA/pF)
NS
Control
FASKard
Time to Reach Maximal I_KATP
Time (min)
NS
Control
FASKard
Control (n=6)
FASKard (n=6)
B
Voltage (mV)
I_Ca (pA/pF)
Control (n=18)
FASKard (n=19)
(Basal current)
+Isoproterenol
Voltage (mV)
I_Ca (pA/pF)
Control (n=9)
FASKard (n=8)
(+Isoproterenol)
C
Cell Capacitance
Cm (pF)
NS
Control
FASKard
Control (n=15)
FASKard (n=21)
D
I_peak
I_peak(pA/pF)
Control (n=7)
FASKard (n=3)
Voltage (mV)
E
I_k,slow
I_k,slow (pA/pF)
Control (n=7)
FASKard (n=3)
Voltage (mV)
F
I_to,fast
I_to,fast (pA/pF)
Control (n=7)
FASKard (n=3)
Voltage (mV)
G
I_ss
I_ss (pA/pF)
Control (n=7)
FASKard (n=5)
Vm (mV)
H
I_K1
Voltage (mV)
I_K1 (pA/pF)
Control (n=7)
FASKard (n=5)


A
Control
FASKard
Mouse #1 #2 #3 #4 #5 #6
LTCC (Cav1.2)
RYR
SERCA2a
B
Control
FASKard
Mouse #1 #2 #3 #4 #5 #6
P-Thr17 PLB
P-Ser16 PLB
Total PLB
C
Control
FASKard
Mouse #1 #2 #3 #4 #5 #6
P-Thr286 CaMKII
Total CaMKIIδ
D
ctrl shRNA
FAS shRNA
FAS
P-Thr286 CaMKII
Total CaMKIIδ
Phospho-CaMKII
Ctrl
FAS shRNA
p<0.05
*
Fold Response (over control shRNA)
2.5
2.0
1.5
1.0
0.5
0.0
Ctrl
FAS shRNA


A
Long-term survival
Percent survival
100
80
60
40
20
0
0
100
200
300
400
500
Days
*p=0.0067
Flox (n=10)
Cre+ (n=8)
FASKard (n=11)
B
Control (Cre+)
FASKard
Mouse (1 yr old)
#1
#2
#3
#4
#5
#6
P-Thr286 CaMKII
Total CaMKII
1 mm


Post-TAC
(+/- CAMKII inhibition)
Percent survival
100
80
60
40
20
0
0
60
120
180
Minutes
KN-93 (n=18)
Control (n=14)
*p=0.015


Mouse
A
WT
(4 weeks old)
ACS Tg
(4 weeks old)
Mouse #1 #2 #3 #1 #2 #3
FAS
β-Actin
WT
(8 weeks old)
ACS Tg
(8 weeks old)
Mouse #1 #2 #3 #1 #2 #3
FAS
β-Actin
B
WT
TRAF2 Tg
Mouse #1 #2 #3 #1 #2 #3
FAS
α-Tubulin
Human
C
FAS mRNA
p=0.026
*
Normal (n=4)
CM (n=5)
Fold over Control
0
2
4
6
8
10
Normal
CM
D
Normal
CM
Patient #1 #2 #1 #2
FAS
α-Tubulin
Patient #1 #2
LVAD pre post pre post
FAS
α-Tubulin


A
Mouse heart
Ctrl IgG
FAS IgG
IP:
IB:
LTCC
(Cav1.2)
Lysate:
LTCC
(Cav1.2)
FAS
B
Voltage (mV)
-45 -35 -25 -15 -5 5 15 25 35
0
-5
-10
-15
-20
I_{Ca} (pA/pF)
BSA alone (n=5)
Palmitate:BSA (n=5)
(Basal current)

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CaMKII-Dependent Diastolic SR Ca^{2+} Leak and Elevated Diastolic Ca^{2+} Levels in Right Atrial Myocardium of Patients With Atrial Fibrillation

Stefan Neef, Nataliya Dybkova, Samuel Sossalla, Katharina R. Ort, Nina Fluschnik, Kay Neumann, Ralf Seipelt, Friedrich A. Schöndube, Gerd Hasenfuss and Lars S. Maier

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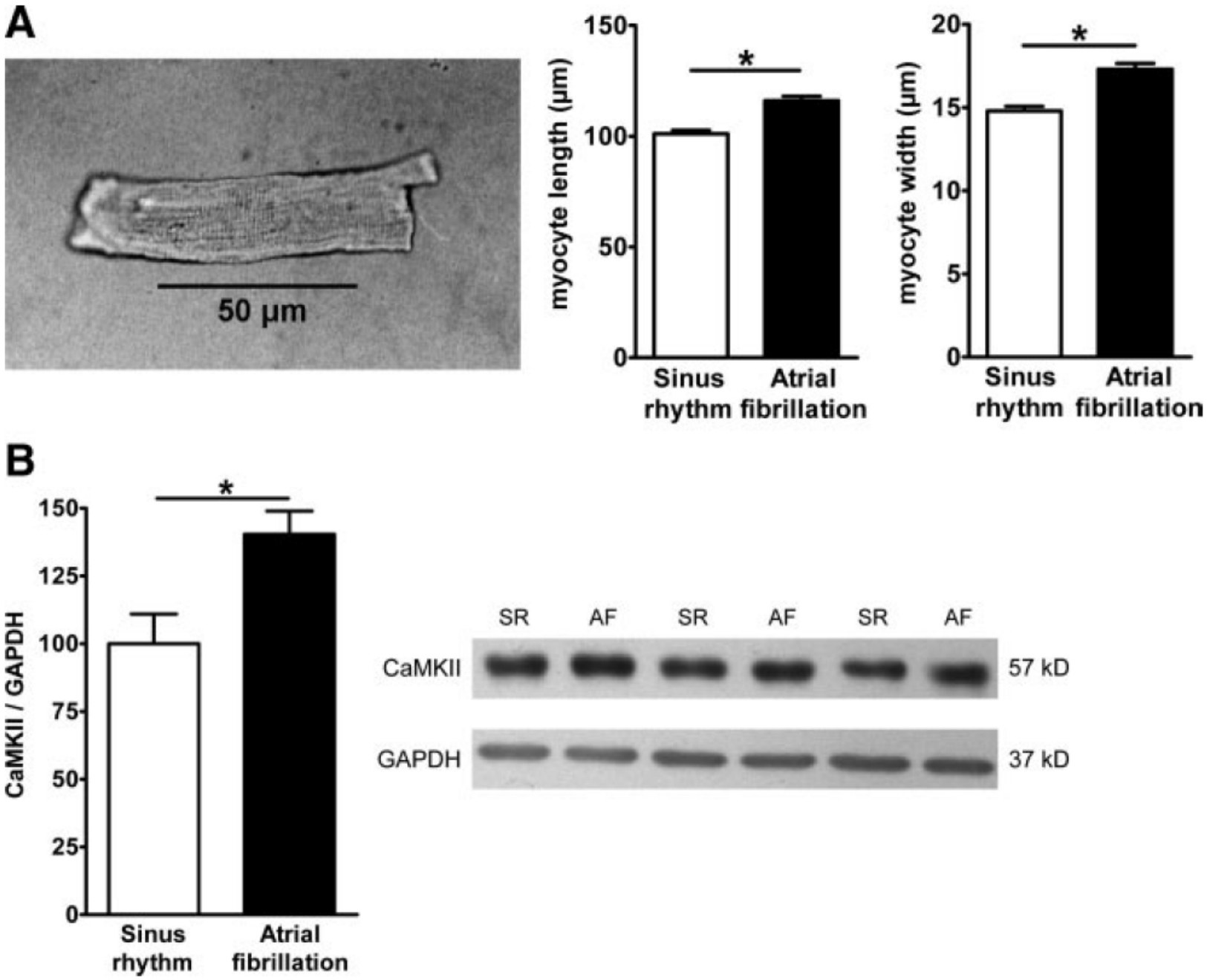
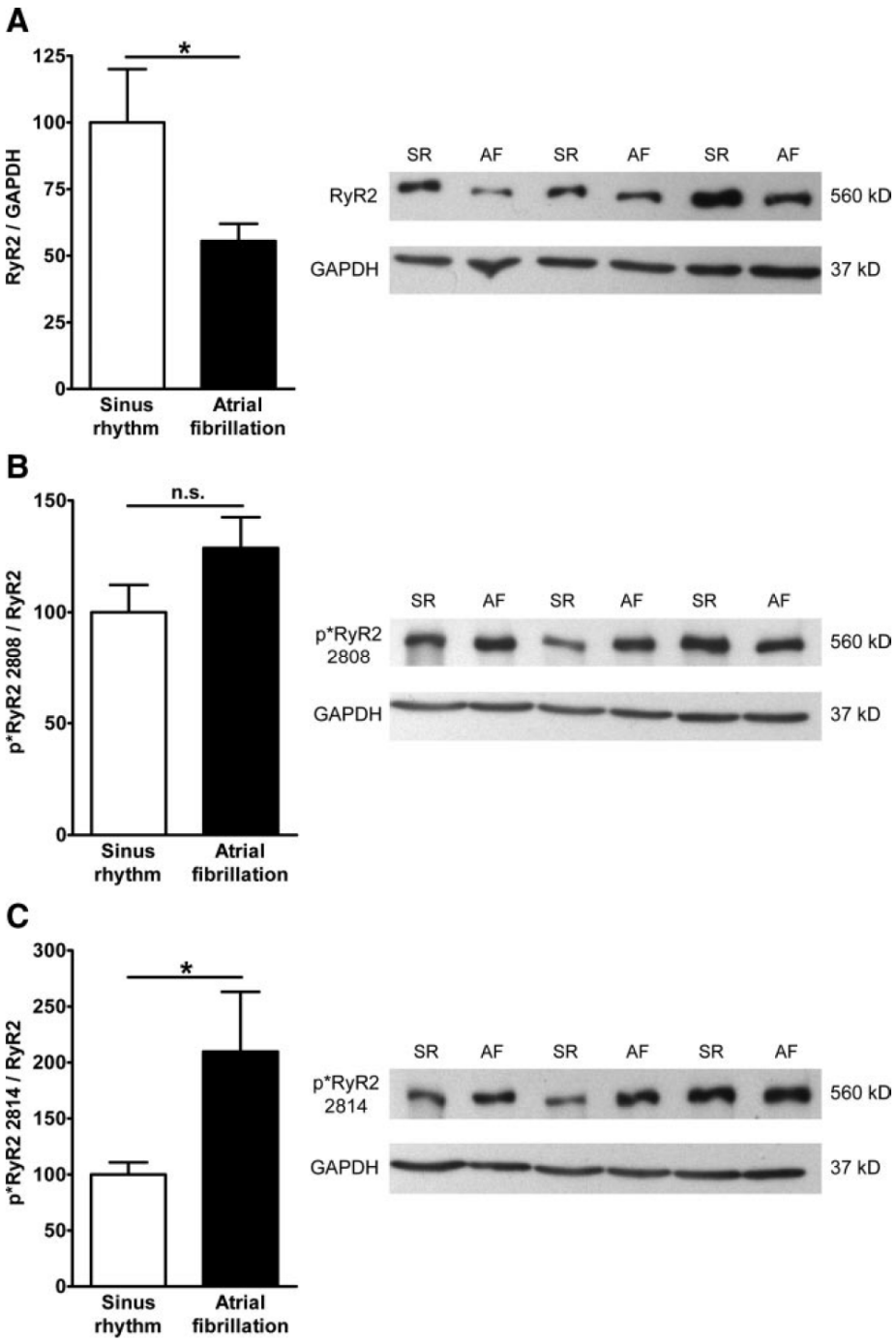
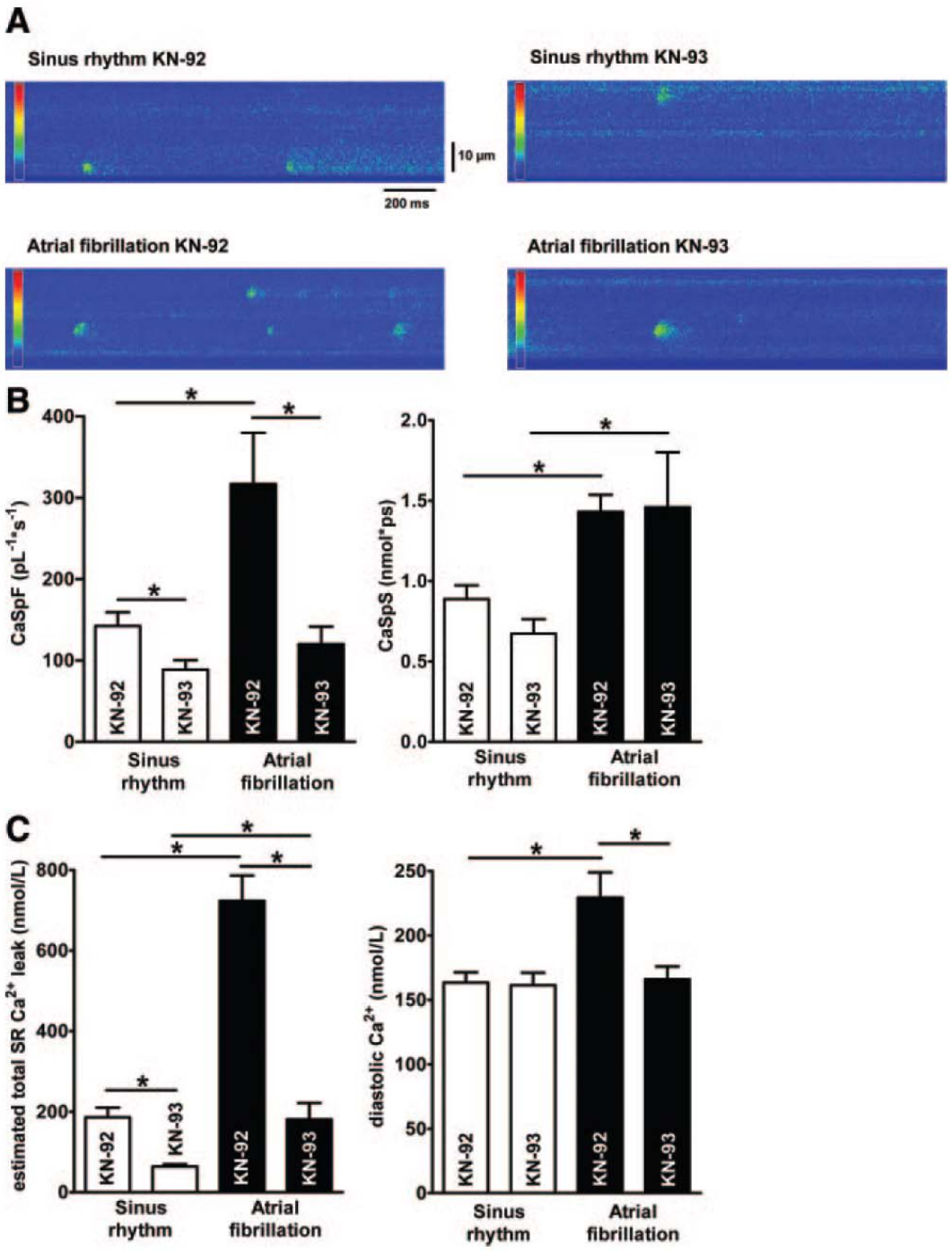
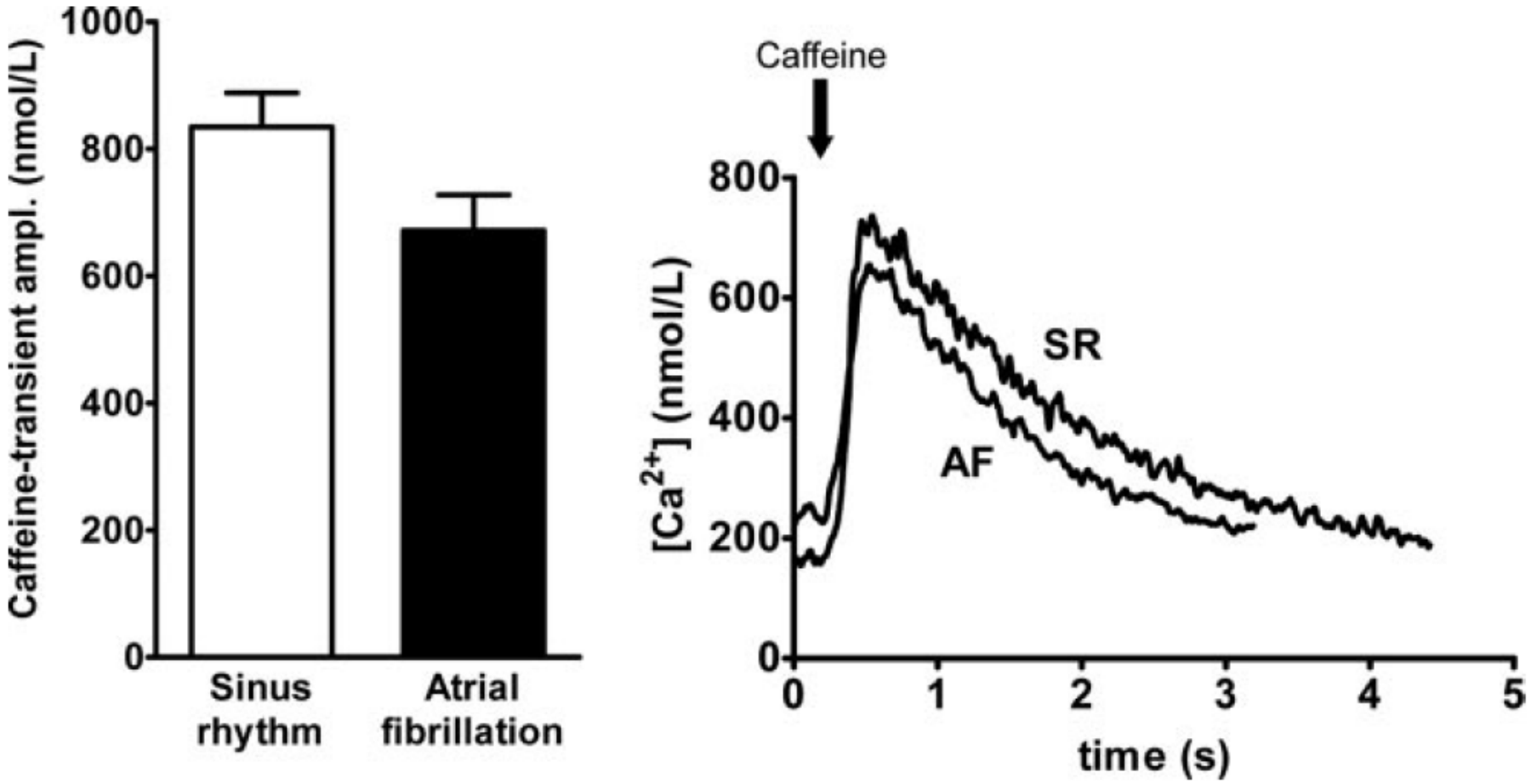
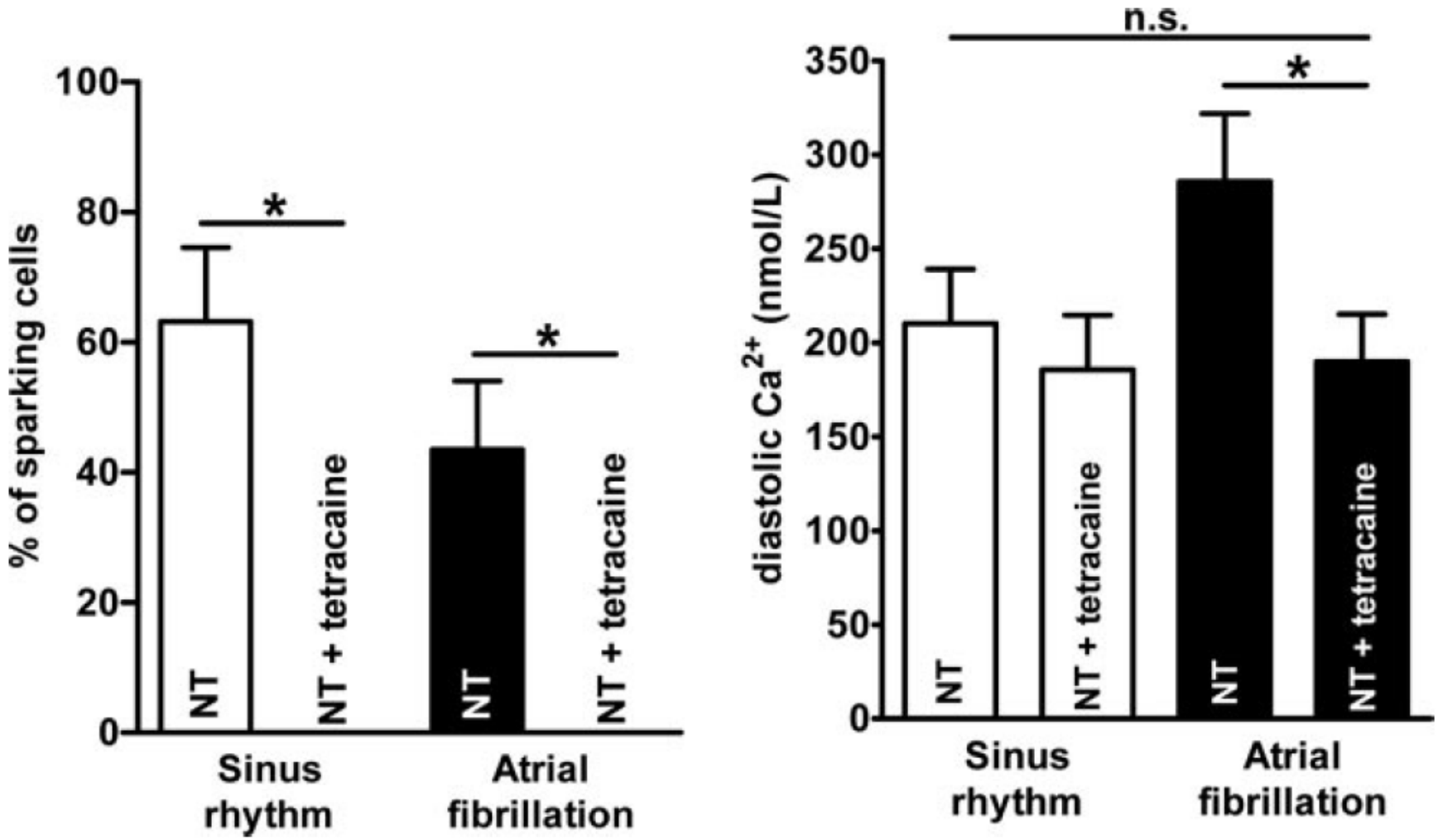
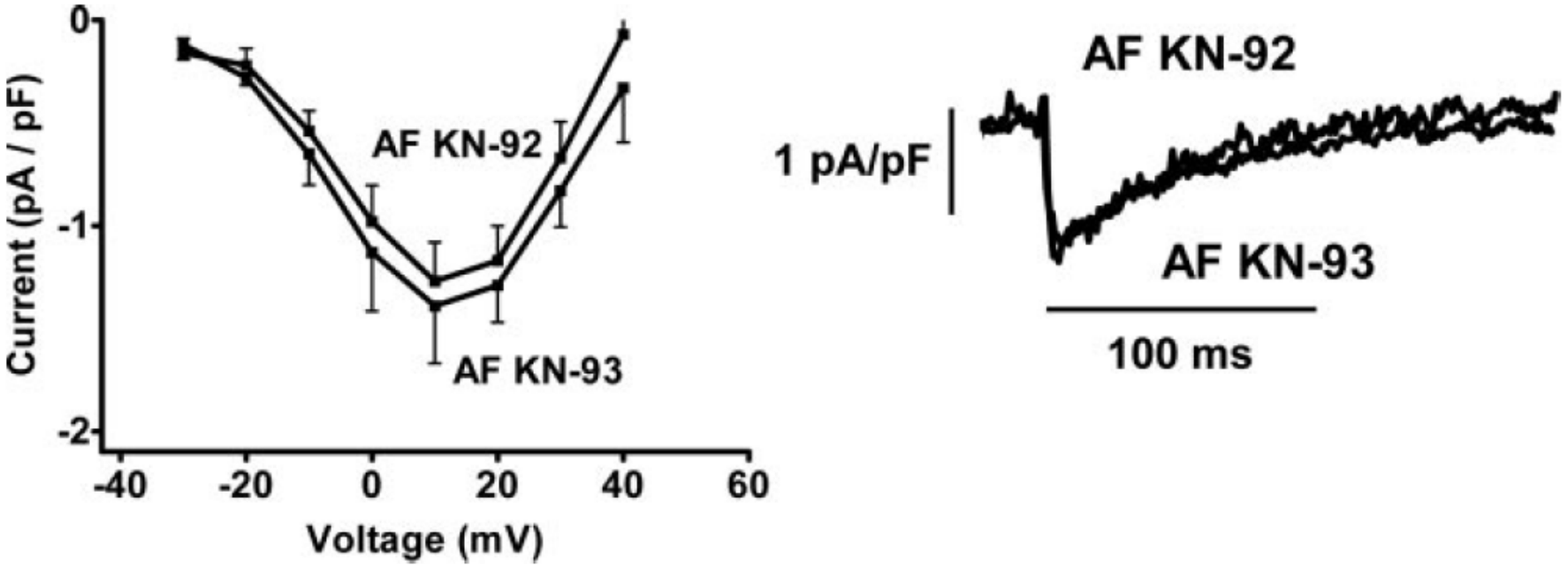

A
50 µm
myocyte length (µm)
150
100
50
0
*
Sinus rhythm
Atrial fibrillation
myocyte width (µm)
20
15
10
5
0
*
Sinus rhythm
Atrial fibrillation
B
CaMKII / GAPDH
150
125
100
75
50
25
0
*
Sinus rhythm
Atrial fibrillation
SR
AF
SR
AF
SR
AF
CaMKII
57 kD
GAPDH
37 kD

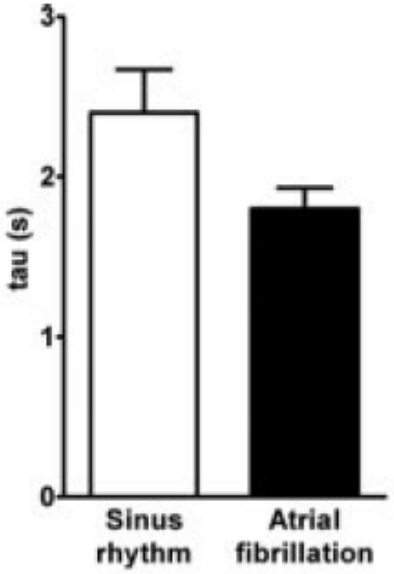

A
125
100
75
50
25
0
RyR2 / GAPDH
*
Sinus rhythm
Atrial fibrillation
SR AF SR AF SR AF
RyR2
560 kD
GAPDH
37 kD
B
150
100
50
0
p*RyR2 2808 / RyR2
n.s.
Sinus rhythm
Atrial fibrillation
SR AF SR AF SR AF
p*RyR2 2808
560 kD
GAPDH
37 kD
C
300
250
200
150
100
50
0
p*RyR2 2814 / RyR2
*
Sinus rhythm
Atrial fibrillation
SR AF SR AF SR AF
p*RyR2 2814
560 kD
GAPDH
37 kD

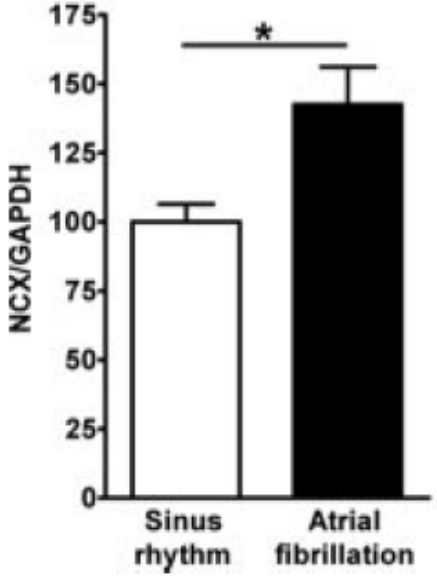

A
Sinus rhythm KN-92
Sinus rhythm KN-93
10 µm
200 ms
Atrial fibrillation KN-92
Atrial fibrillation KN-93
B
CaSpF (pL⁻¹s⁻¹)
CaSpS (nmol*ps)
KN-92
KN-93
Sinus rhythm
Atrial fibrillation
C
estimated total SR Ca2+ leak (nmol/L)
diastolic Ca2+ (nmol/L)

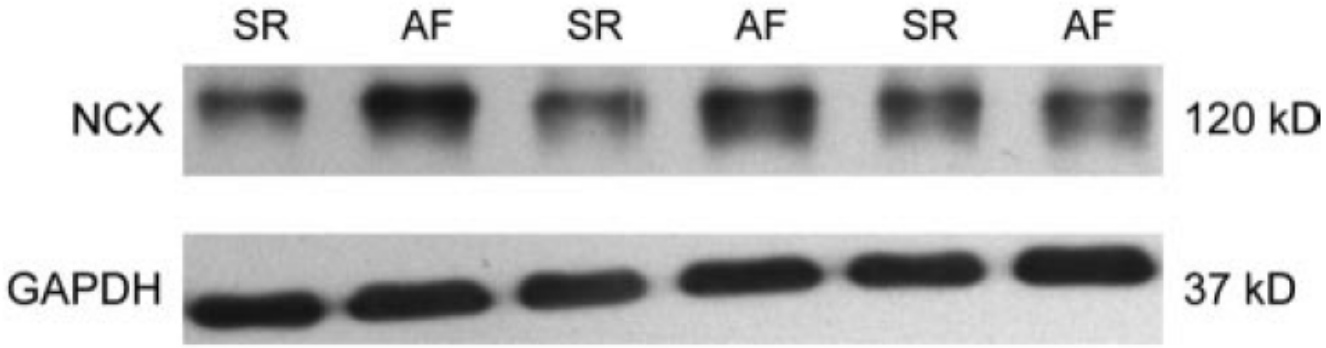

Caffeine-transient ampl. (nmol/L)
1000
800
600
400
200
0
Sinus rhythm
Atrial fibrillation
Caffeine
[Ca2+] (nmol/L)
800
600
400
200
0
SR
AF
0
1
2
3
4
5
time (s)

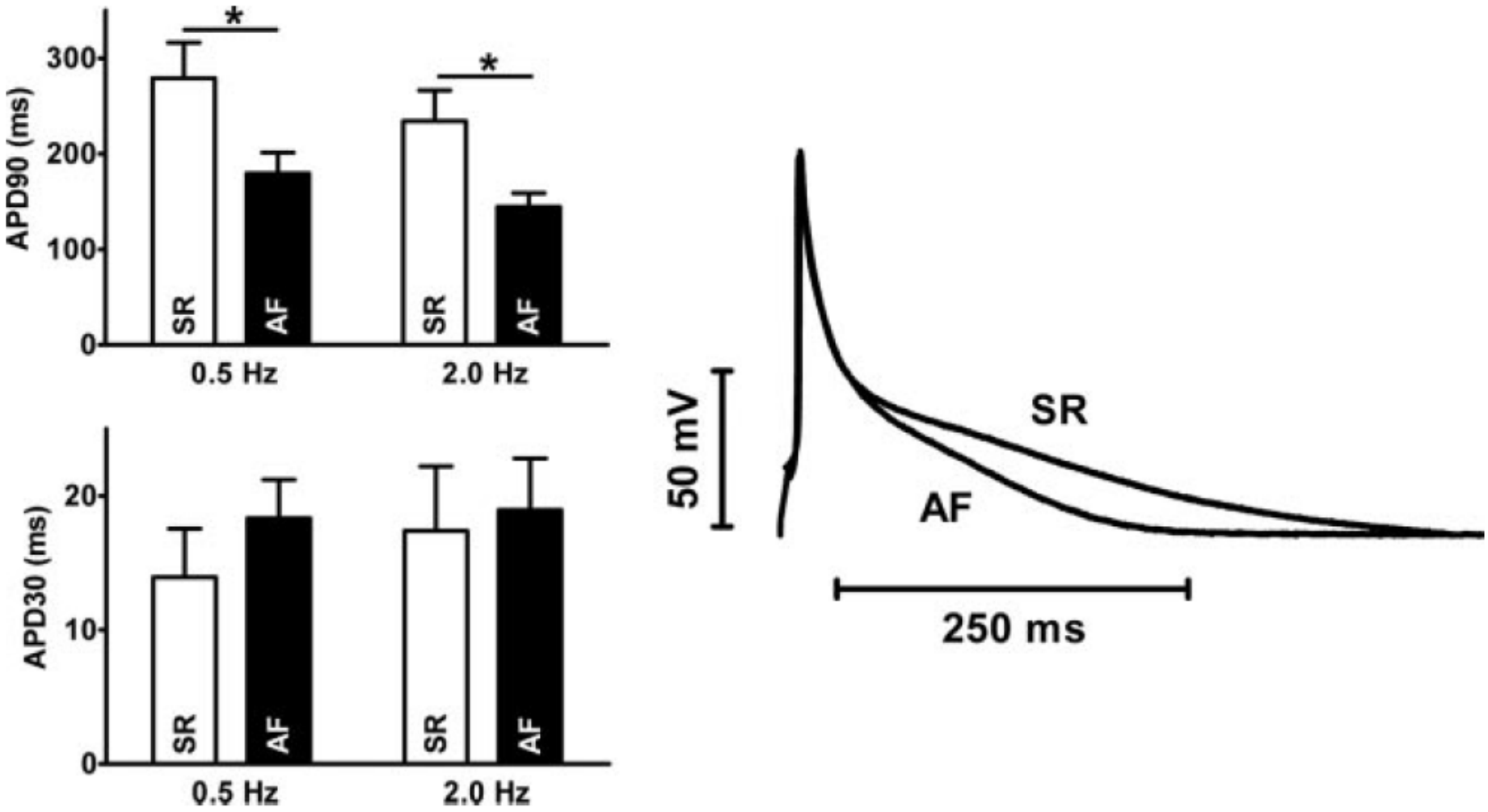

% of sparking cells
100
80
60
40
20
0
*
*
NT
NT + tetracaine
NT
NT + tetracaine
Sinus rhythm
Atrial fibrillation
n.s.
*
diastolic Ca2+ (nmol/L)
350
300
250
200
150
100
50
0
NT
NT + tetracaine
NT
NT + tetracaine
Sinus rhythm
Atrial fibrillation


0
-1
-2
Current (pA / pF)
AF KN-92
AF KN-93
-40
-20
0
20
40
60
Voltage (mV)
AF KN-92
1 pA/pF
AF KN-93
100 ms


3
2
1
0
tau (s)
Sinus rhythm
Atrial fibrillation


175
150
125
100
75
50
25
0
NCX/GAPDH
*
Sinus rhythm
Atrial fibrillation


SR
AF
SR
AF
SR
AF
NCX
120 kD
GAPDH
37 kD


APD90 (ms)
300
200
100
0
*
*
SR
AF
SR
AF
0.5 Hz
2.0 Hz
APD30 (ms)
20
10
0
SR
AF
SR
AF
0.5 Hz
2.0 Hz
50 mV
SR
AF
250 ms

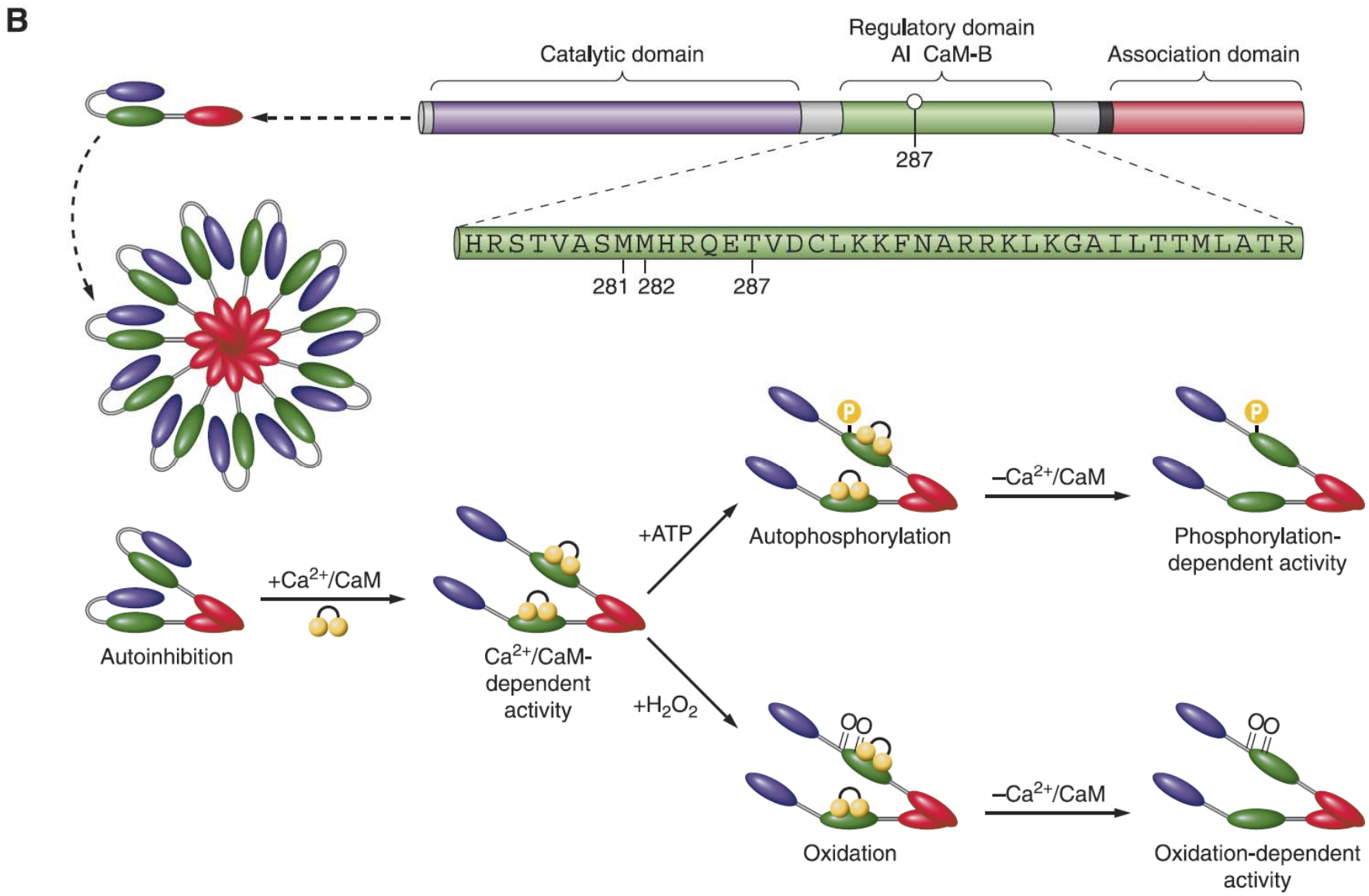
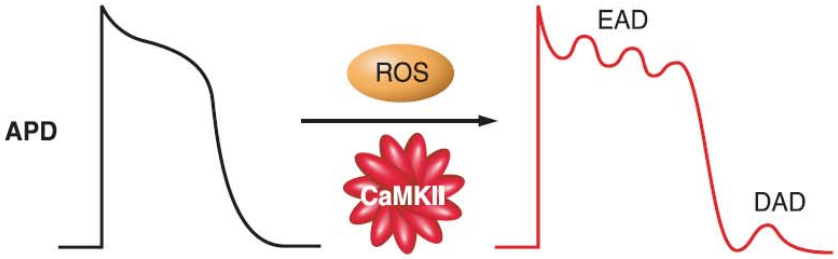
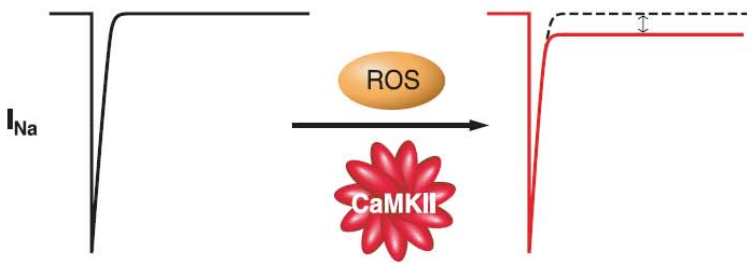
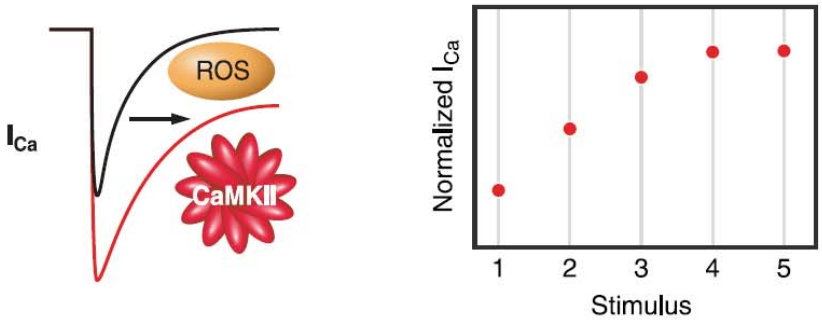


FIGURE 1 Oxidation and autophosphorylation both convert CaMKII into a Ca^{2+}/CaM-independent enzyme by modification of defined CaMKII regulatory domain amino acids.



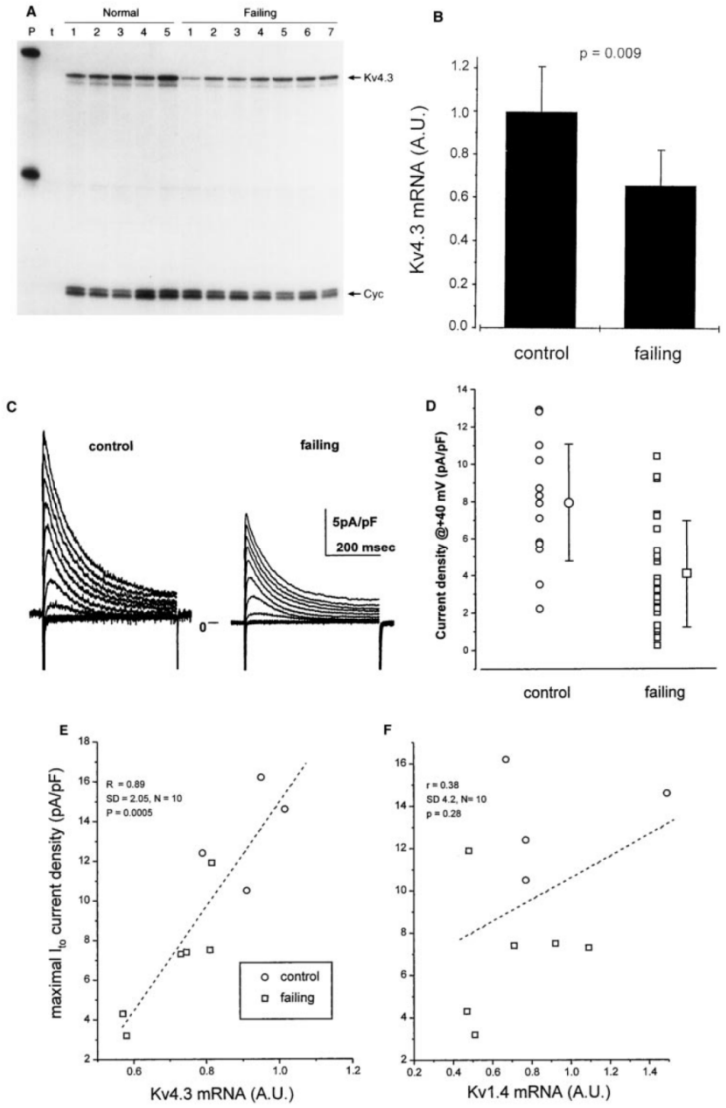


- increase slowly inactivating current
- increase subsarcolemma [Na]
- reduce Ca^{2+} efflux via Na/Ca exchanger
- APD prolongation and EADs
- raise intracellular [Ca] and DADs



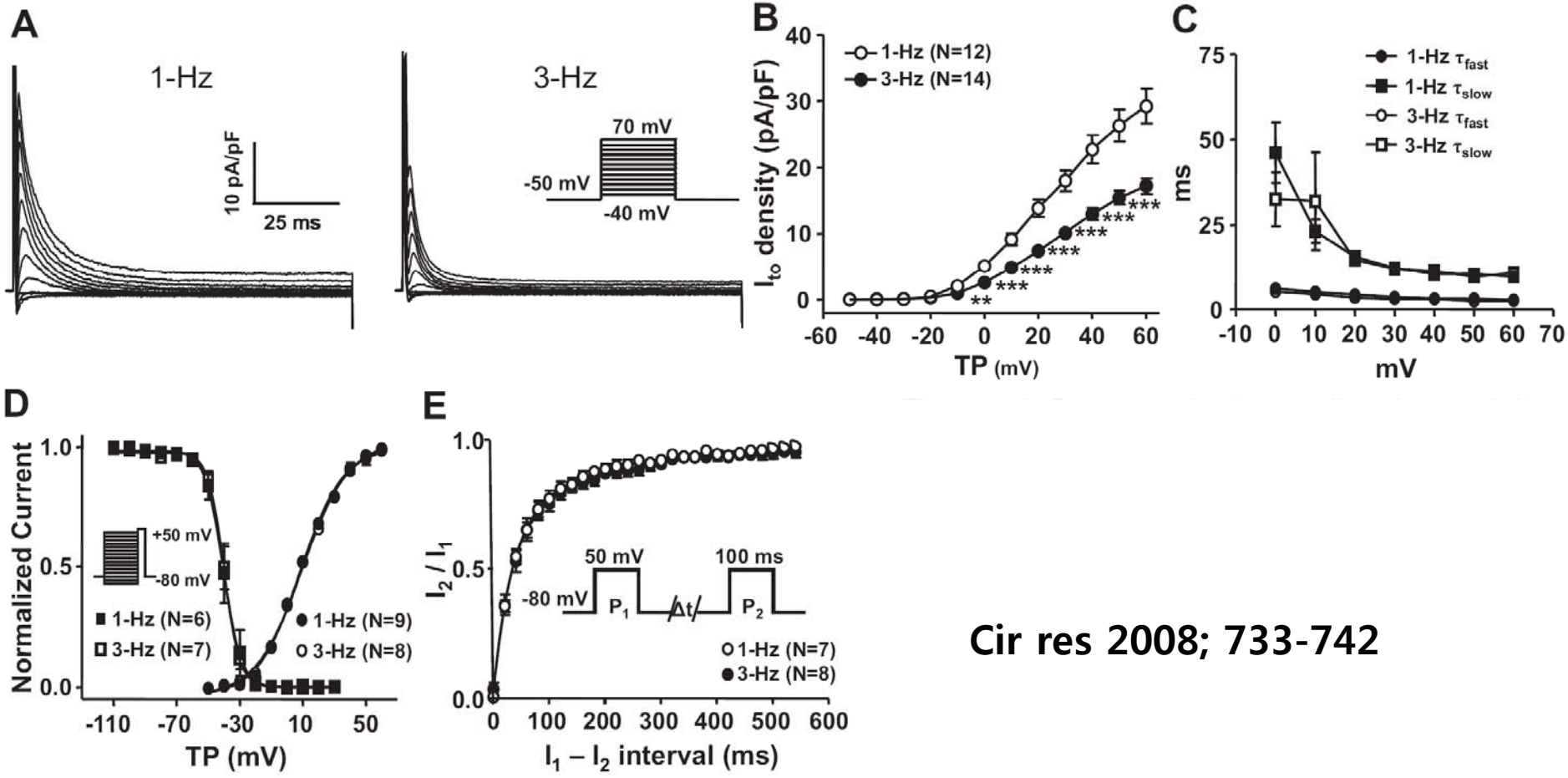
- I_{Ca} facilitation: increase peak current and slow inactivation
- APD prolongation and EADs
- raise intracellular [Ca] and DADs

Decreased I_{to} in CHF

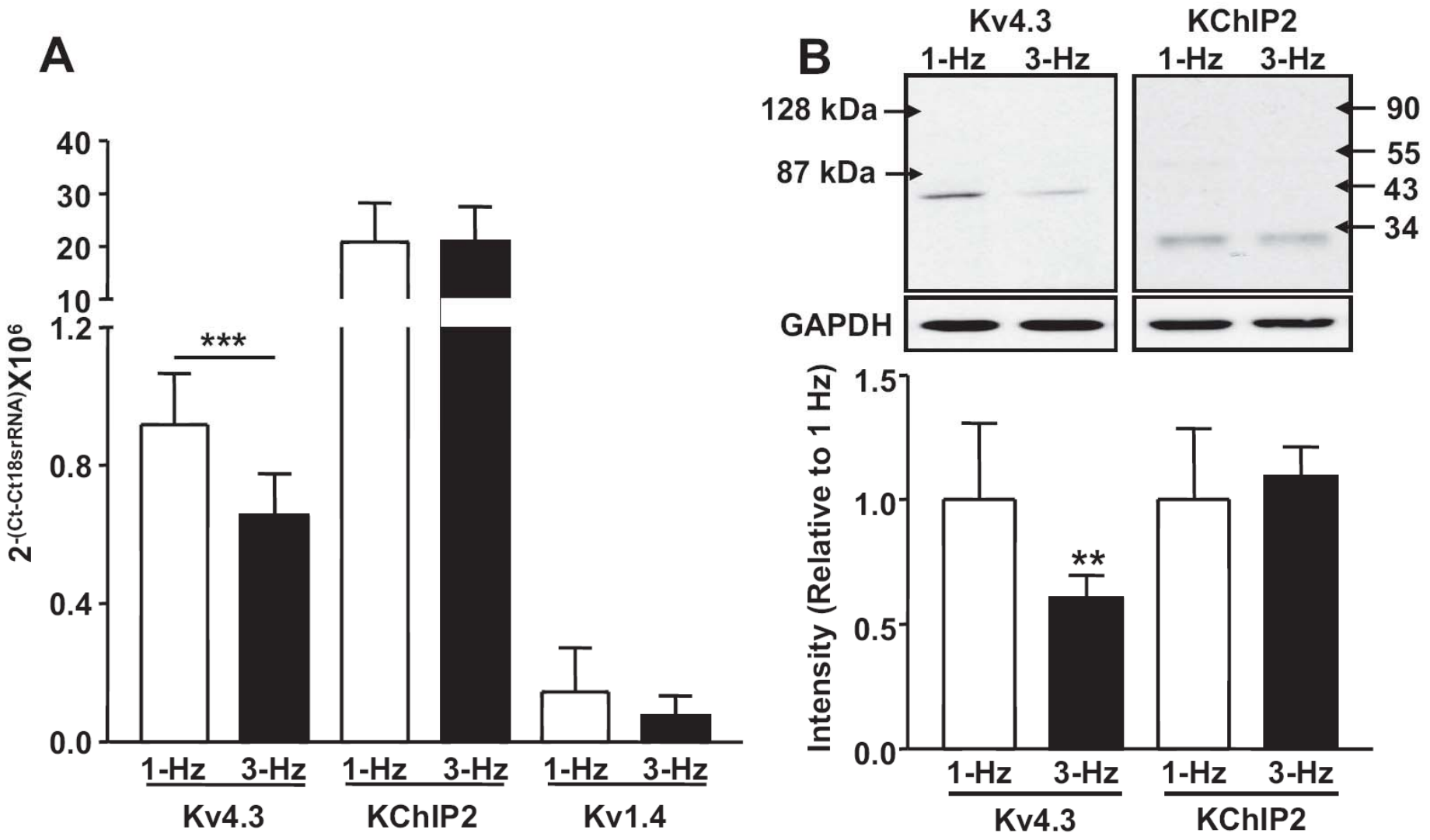


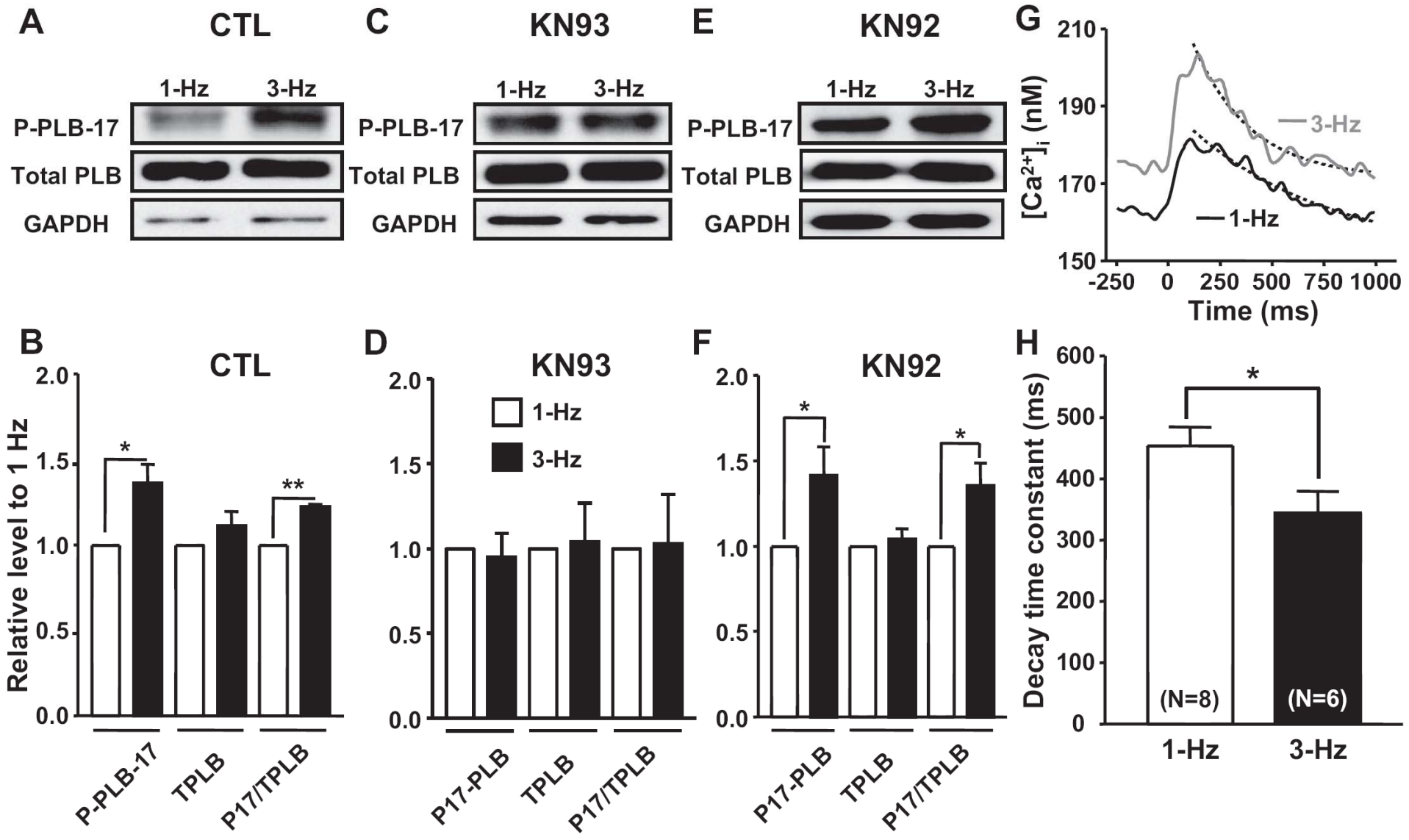
Cir 1998; 1383-1393

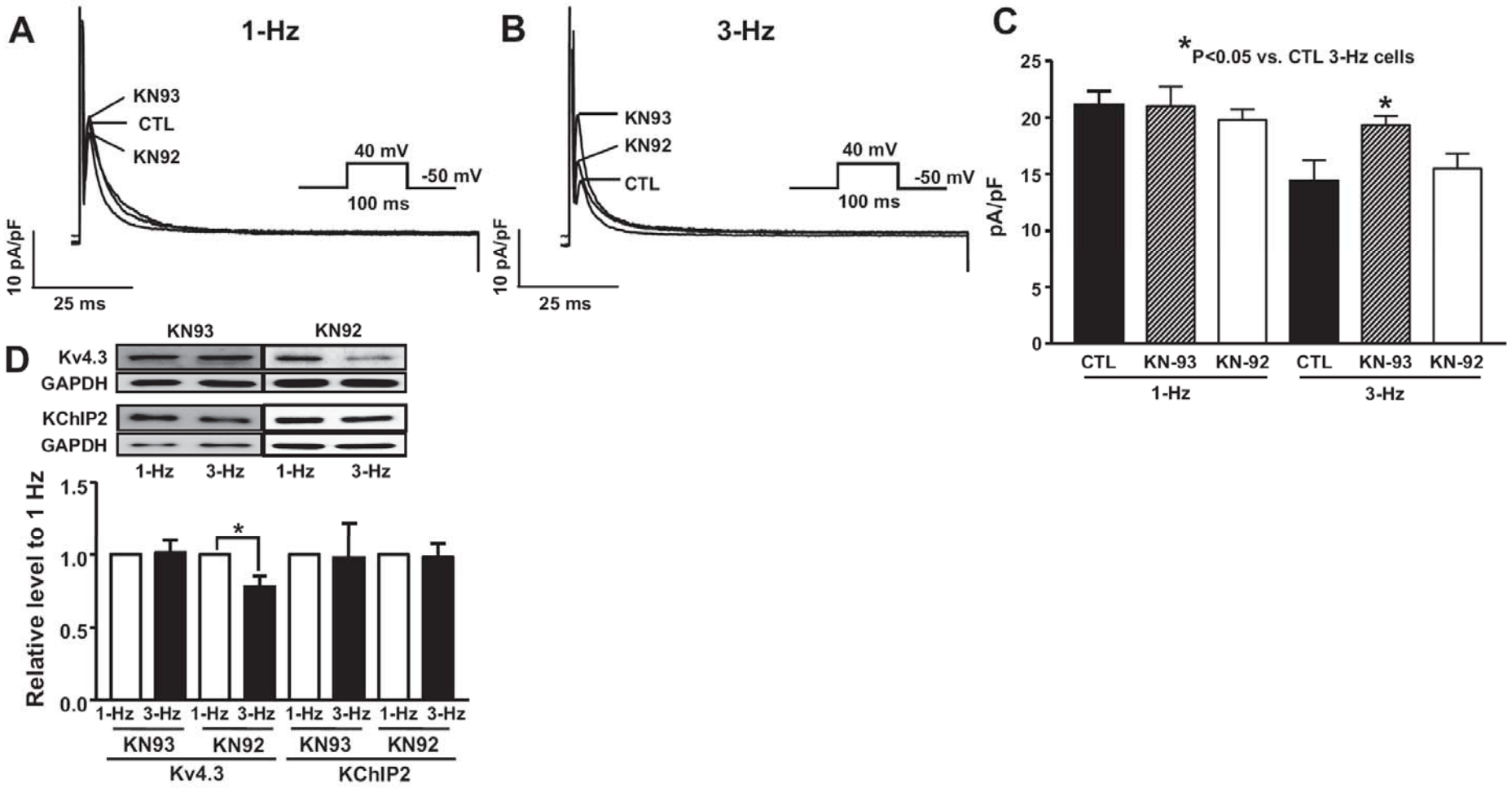
Mechanism underlying rate dependent remodeling of Ito in canine ventricular myocytes

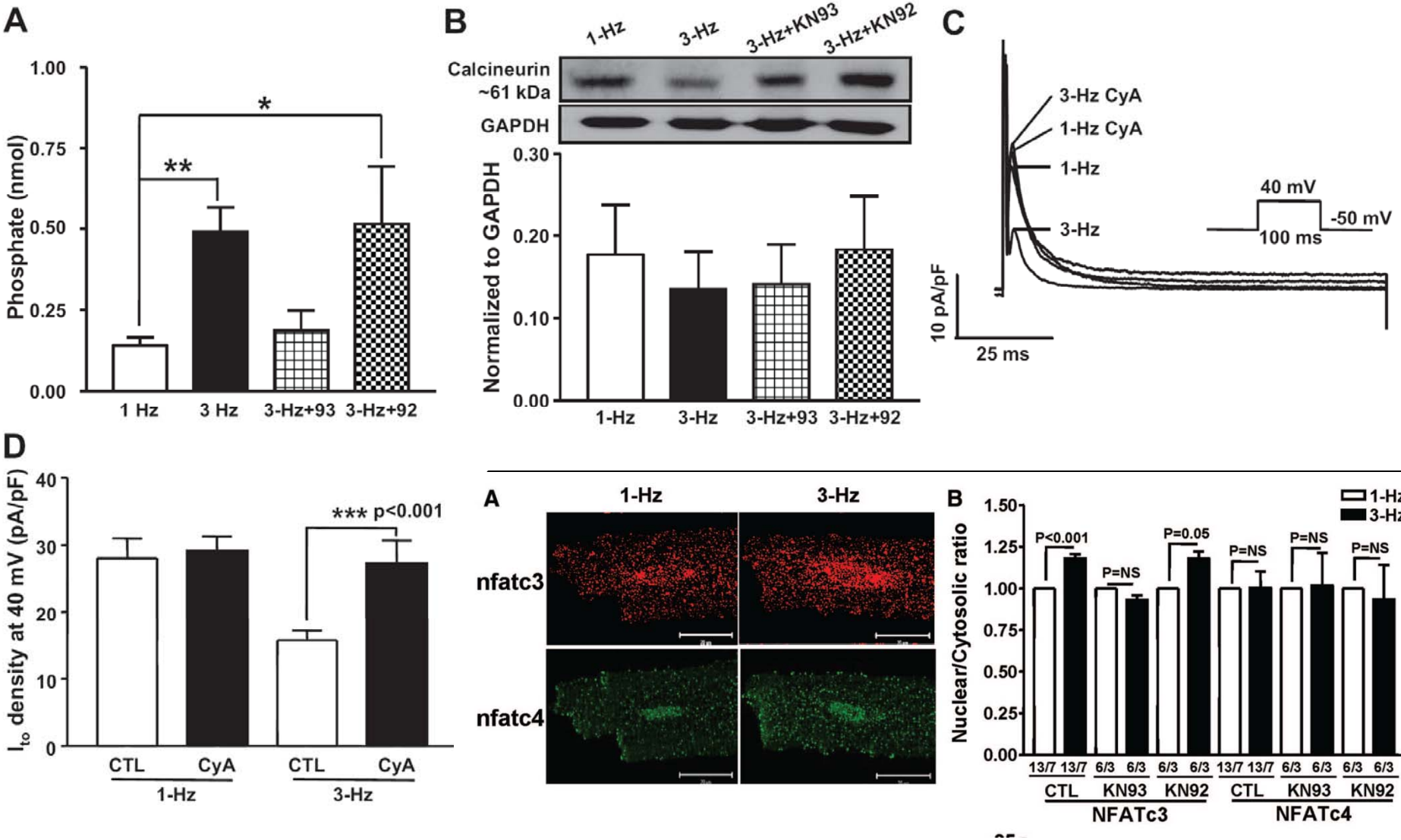


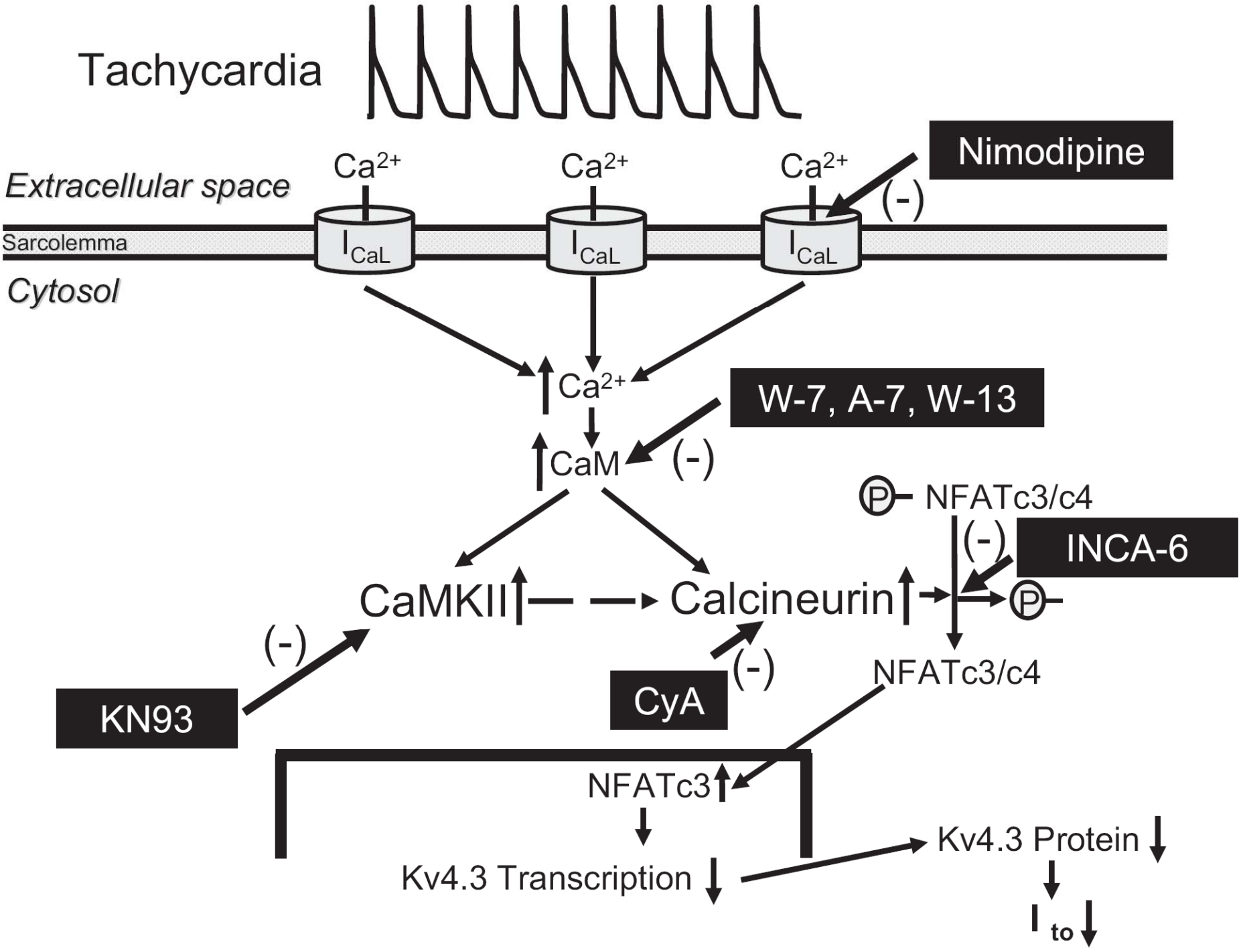
Cir res 2008; 733-742


A
40
30
20
10
1.2
0.8
0.4
0.0
2-(Ct-Ct18srRNA)X10^6
***
1-Hz
3-Hz
Kv4.3
KChIP2
Kv1.4
B
Kv4.3
KChIP2
1-Hz
3-Hz
128 kDa
87 kDa
90
55
43
34
GAPDH
Intensity (Relative to 1 Hz)
1.5
1.0
0.5
0.0
**


A
CTL
1-Hz
3-Hz
P-PLB-17
Total PLB
GAPDH
C
KN93
1-Hz
3-Hz
P-PLB-17
Total PLB
GAPDH
E
KN92
1-Hz
3-Hz
P-PLB-17
Total PLB
GAPDH
G
210
190
170
150
[Ca2+]i (nM)
3-Hz
1-Hz
-250
0
250
500
750
1000
Time (ms)
B
CTL
Relative level to 1 Hz
2.0
1.5
1.0
0.5
0.0
*
**
P-PLB-17
TPLB
P17/TPLB
D
KN93
1-Hz
3-Hz
2.0
1.5
1.0
0.5
0.0
P17-PLB
TPLB
P17/TPLB
F
KN92
2.0
1.5
1.0
0.5
0.0
*
*
P17-PLB
TPLB
P17/TPLB
H
Decay time constant (ms)
600
500
400
300
200
100
0
*
(N=8)
(N=6)
1-Hz
3-Hz


A
1-Hz
KN93
CTL
KN92
40 mV
-50 mV
100 ms
10 pA/pF
25 ms
B
3-Hz
KN93
KN92
CTL
40 mV
-50 mV
100 ms
10 pA/pF
25 ms
C
*P<0.05 vs. CTL 3-Hz cells
pA/pF
25
20
15
10
5
0
CTL
KN-93
KN-92
CTL
KN-93
KN-92
1-Hz
3-Hz
D
KN93
KN92
Kv4.3
GAPDH
KChIP2
GAPDH
1-Hz
3-Hz
1-Hz
3-Hz
Relative level to 1 Hz
1.5
1.0
0.5
0.0
1-Hz 3-Hz 1-Hz 3-Hz 1-Hz 3-Hz 1-Hz 3-Hz
KN93
KN92
KN93
KN92
Kv4.3
KChIP2


A
Phosphate (nmol)
1.00
0.75
0.50
0.25
0.00
*
**
1 Hz
3 Hz
3-Hz+93
3-Hz+92
B
1-Hz
3-Hz
3-Hz+KN93
3-Hz+KN92
Calcineurin
~61 kDa
GAPDH
Normalized to GAPDH
0.30
0.20
0.10
0.00
1-Hz
3-Hz
3-Hz+93
3-Hz+92
C
3-Hz CyA
1-Hz CyA
1-Hz
3-Hz
40 mV
-50 mV
100 ms
10 pA/pF
25 ms
D
I to density at 40 mV (pA/pF)
40
30
20
10
0
*** p<0.001
CTL
CyA
CTL
CyA
1-Hz
3-Hz
A
1-Hz
3-Hz
nfatc3
nfatc4
B
1-Hz
3-Hz
Nuclear/Cytosolic ratio
1.50
1.25
1.00
0.75
0.50
0.25
0.00
P<0.001
P=NS
P=0.05
P=NS
P=NS
P=NS
13/7 13/7
6/3 6/3
6/3 6/3
13/7 13/7
6/3 6/3
6/3 6/3
CTL
KN93
KN92
CTL
KN93
KN92
NFATc3
NFATc4


Tachycardia
Extracellular space
Sarcolemma
Cytosol
Ca2+
Ca2+
Ca2+
ICaL
ICaL
ICaL
Nimodipine
(-)
Ca2+
W-7, A-7, W-13
CaM
(-)
P
NFATc3/c4
(-)
INCA-6
CaMKII
Calcineurin
P
(-)
KN93
(-)
CyA
NFATc3/c4
NFATc3
Kv4.3 Transcription
Kv4.3 Protein
Ito

감사합니다.