Genetics in cardiac arrhythmias

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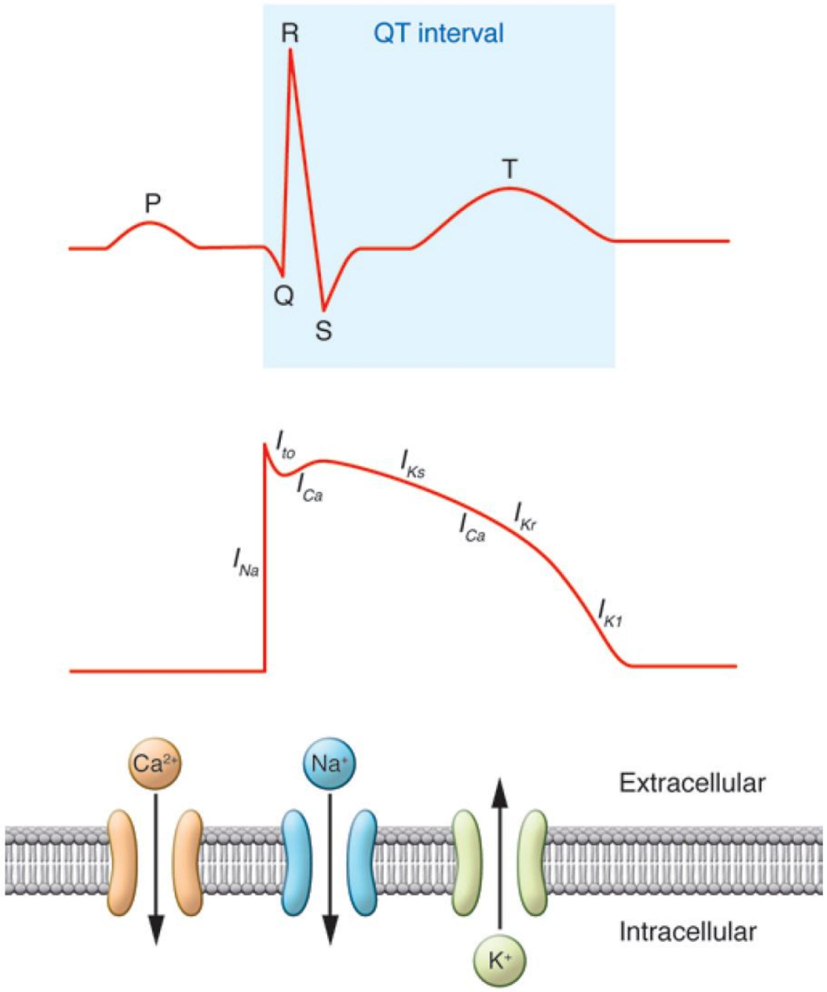
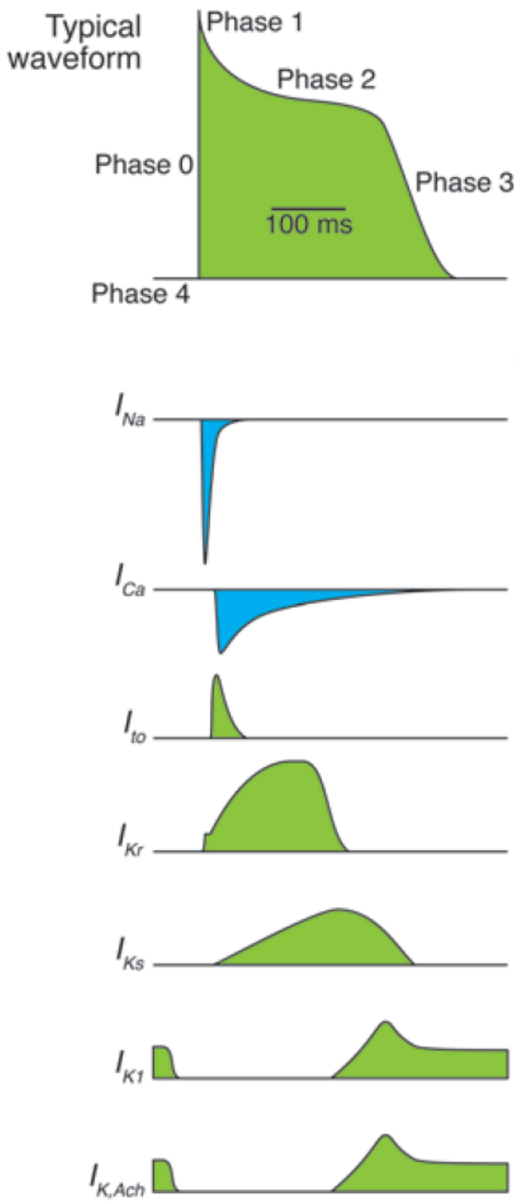
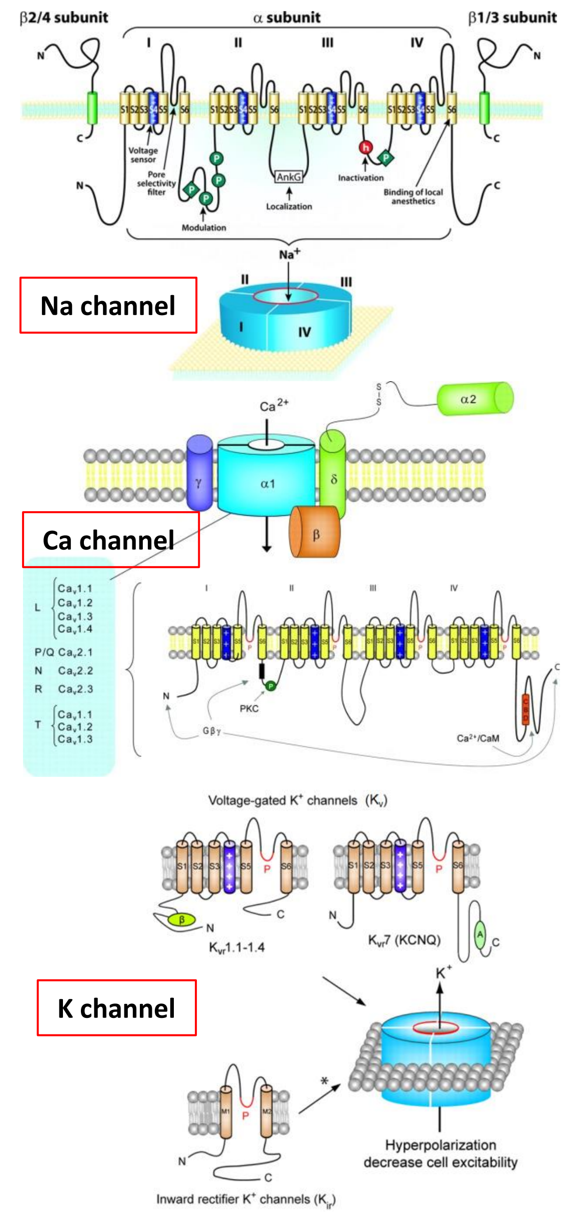
ECG and action potential (AP)



Ionic & molecular basis for AP

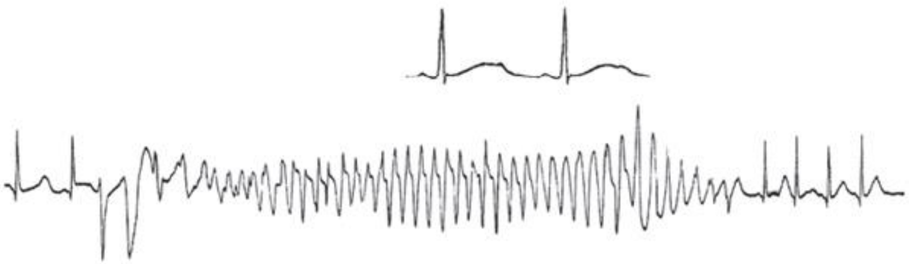


Current	Pore-forming subunits	Auxiliary subunits	Interacting proteins
I_{Na}	$Na_v1.5$ (SCN5A)	β1 (SCN1B) β2 (SCN2B) β3 (SCN3B) β4 (SCN4B)	ANK2 CAV3 GPD1L Syntrophin MOG1
I_{Ca}	$Ca_v1.2$ (CACNA1C)	β2 (CACNB2) $\alpha_2\delta$ (CACNA2D1)	Calmodulin
I_{to}	$K_v4.3$ (KCND3)	KChIP2	KCNE3 KCNE5
I_{Kr}	$K_v11.1$ (HERG)	KCNE2	--
I_{Ks}	$K_v7.1$ (KCNQ1)	--	Yotiao (AKAP9)
I_{K1}	$Kir_v2.1$ (KCNJ2) $Kir_v2.2$ (KCNJ12)	--	--
$I_{K,Ach}$	$Kir_v3.4$ (KCNJ5)	--	G protein βγ

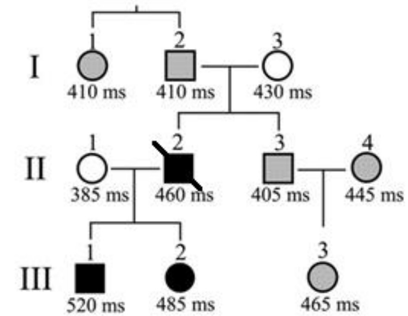


Experiment of inherited arrhythmia (I)

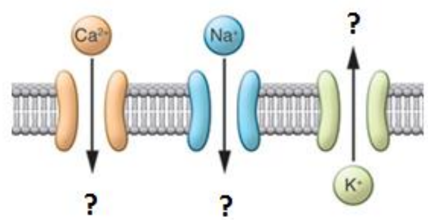
unexplained syncope
sudden cardiac arrest



family history



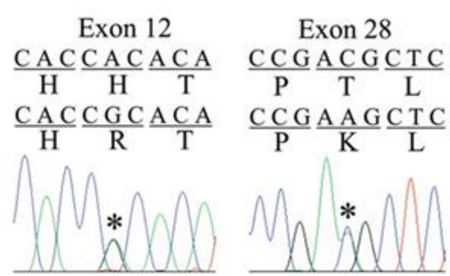
genetic disorder ?



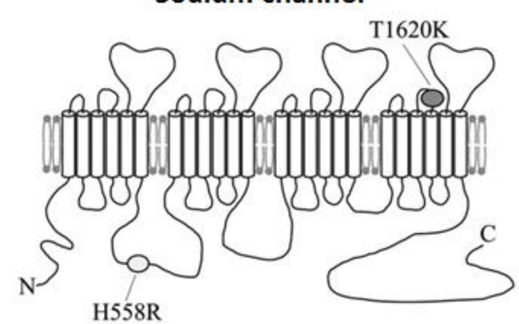
Genetic study

Pore-forming subunits	Auxiliary subunits	Interacting proteins
$Na_v1.5$ (SCN5A)	β1 (SCN1B) β2 (SCN2B) β3 (SCN3B) β4 (SCN4B)	ANK2 CAV3 GPD1L Syntrophin MOG1
$Ca_v1.2$ (CACNA1C)	β2 (CACNB2) $\alpha_2\delta$ (CACNA2D1)	Calmodulin
$K_v4.3$ (KCND3)	KChIP2	KCNE3 KCNE5
$K_v11.1$ (HERG)	KCNE2	--
$K_v7.1$ (KCNQ1)	--	Yotiao (AKAP9)
$Kir_2.1$ (KCNJ2) $Kir_2.2$ (KCNJ12)	--	--
$Kir_3.4$ (KCNJ5)	--	G protein βγ

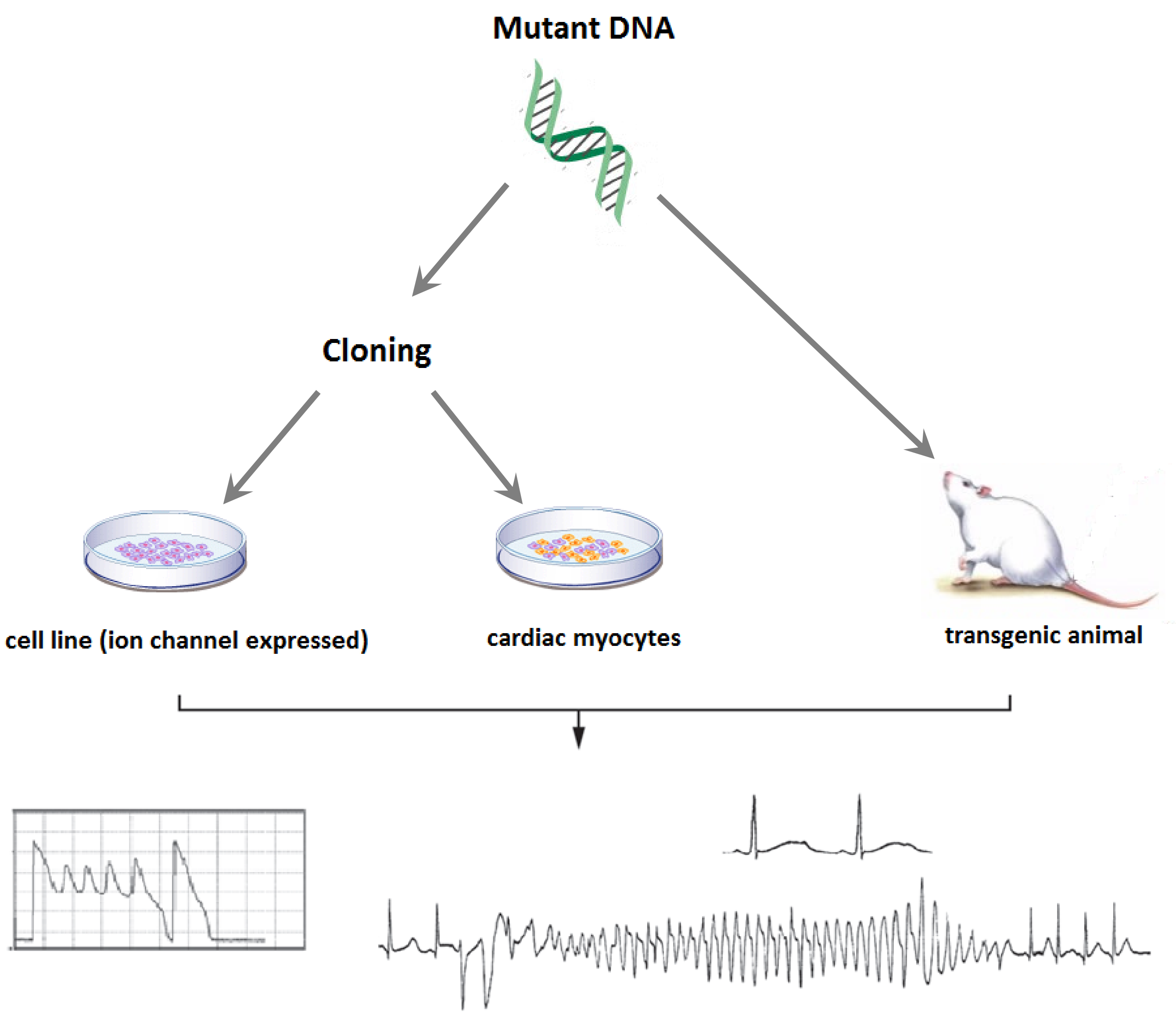
SCN5A



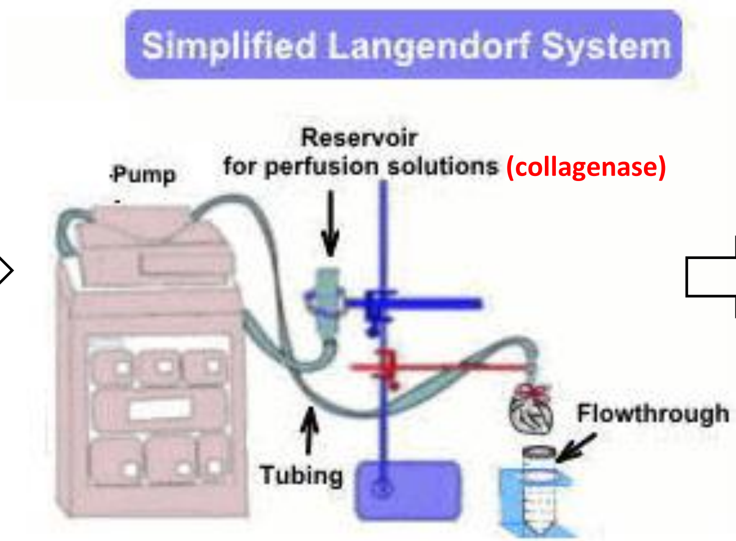
Sodium channel

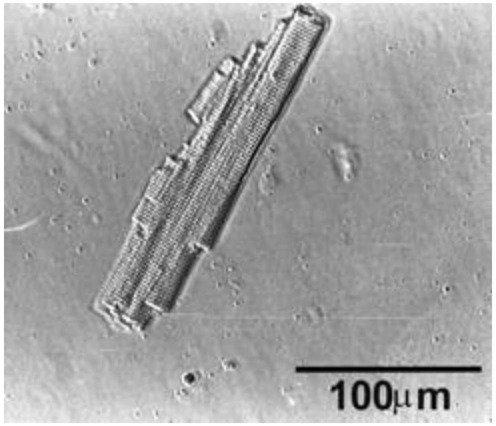


Experiment of inherited arrhythmia (II)

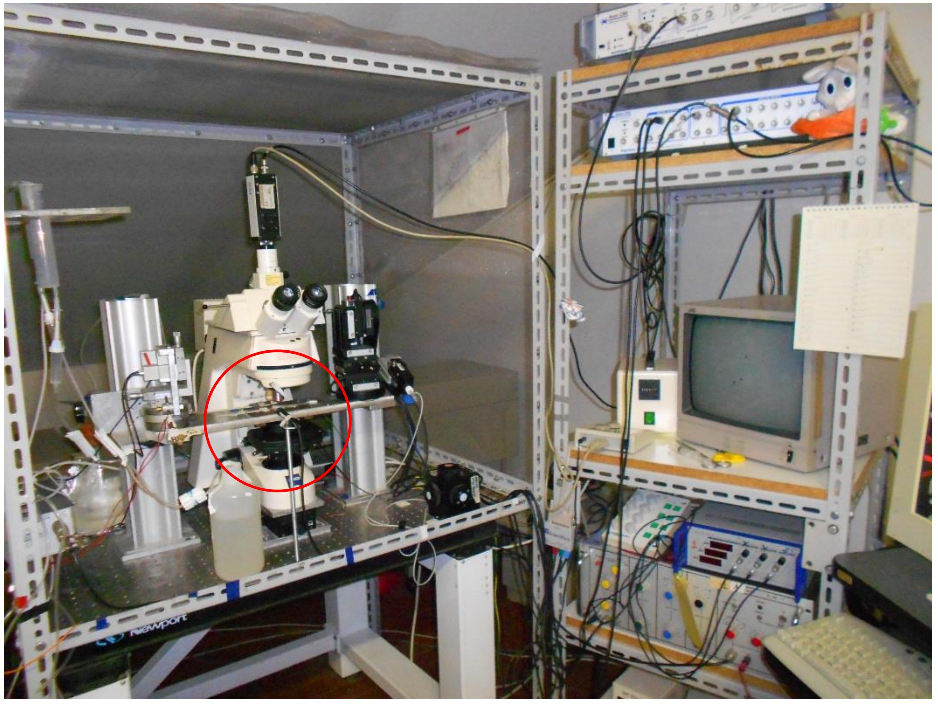


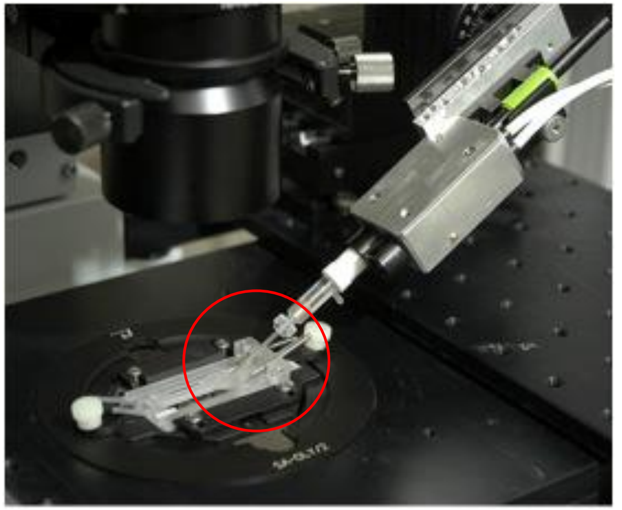
Cardiomyocytes isolation

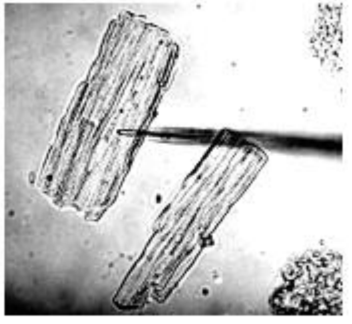


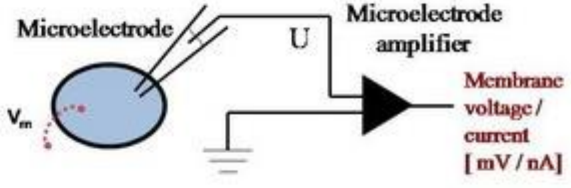


Patch clamp (electrophysiologic study)





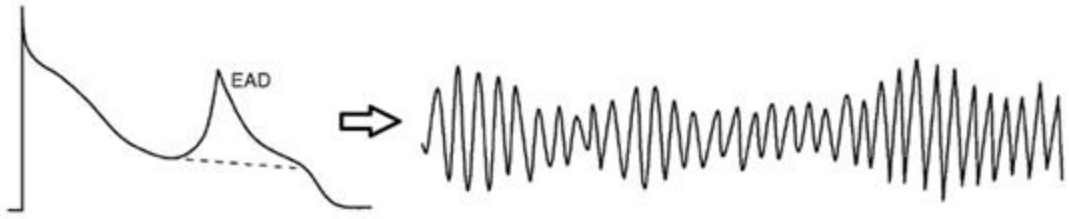


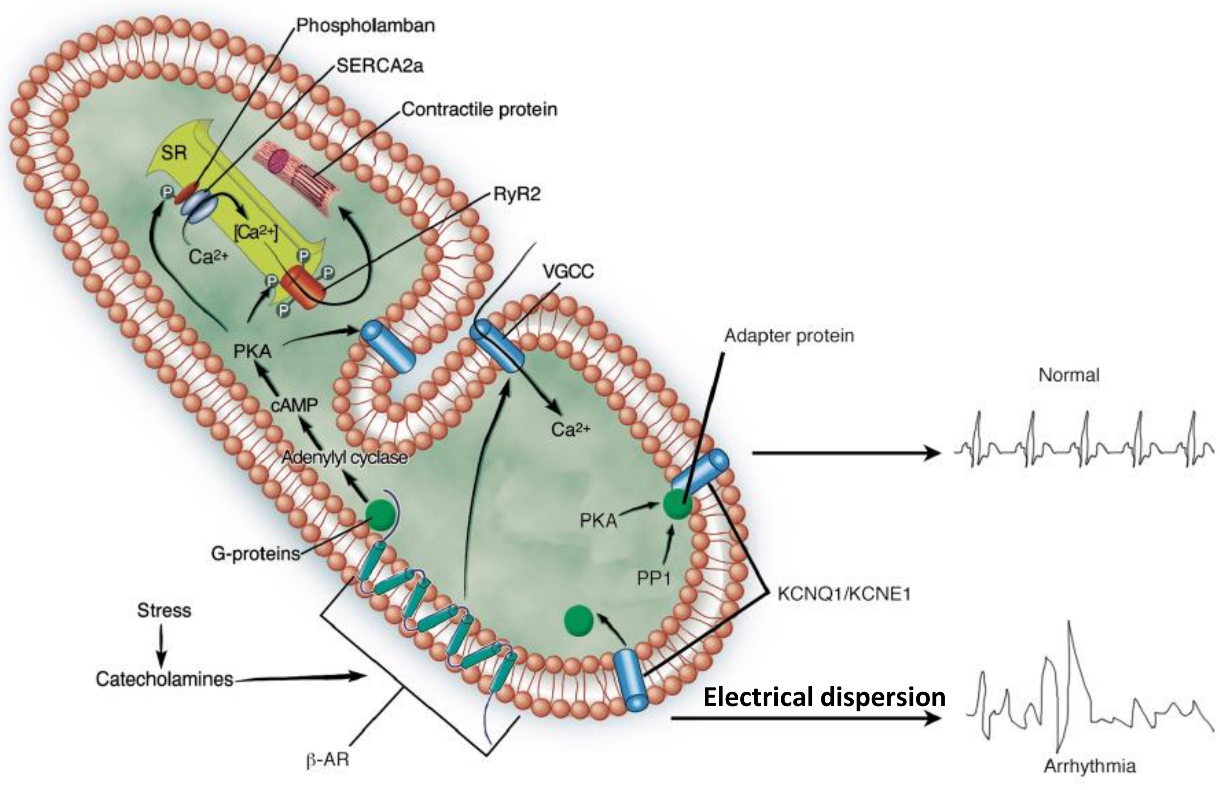


Inherited arrhythmias

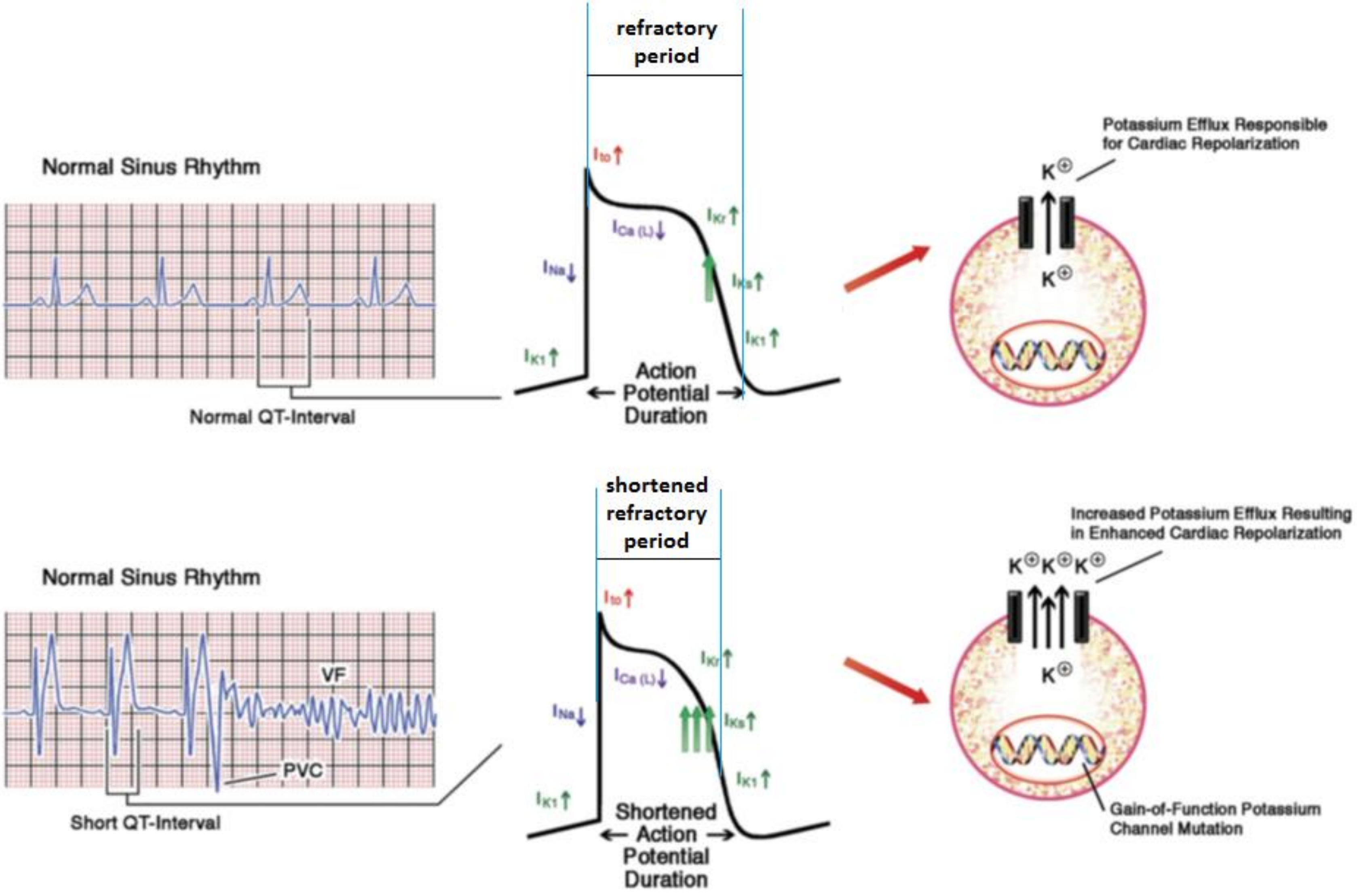
- Long QT syndrome (LQTS)
- Short QT syndrome (SQTS)
- Brugada syndrome
- Catecholaminergic polymorphic ventricular tachycardia (CPVT)

Long QT syndrome (LQTS)

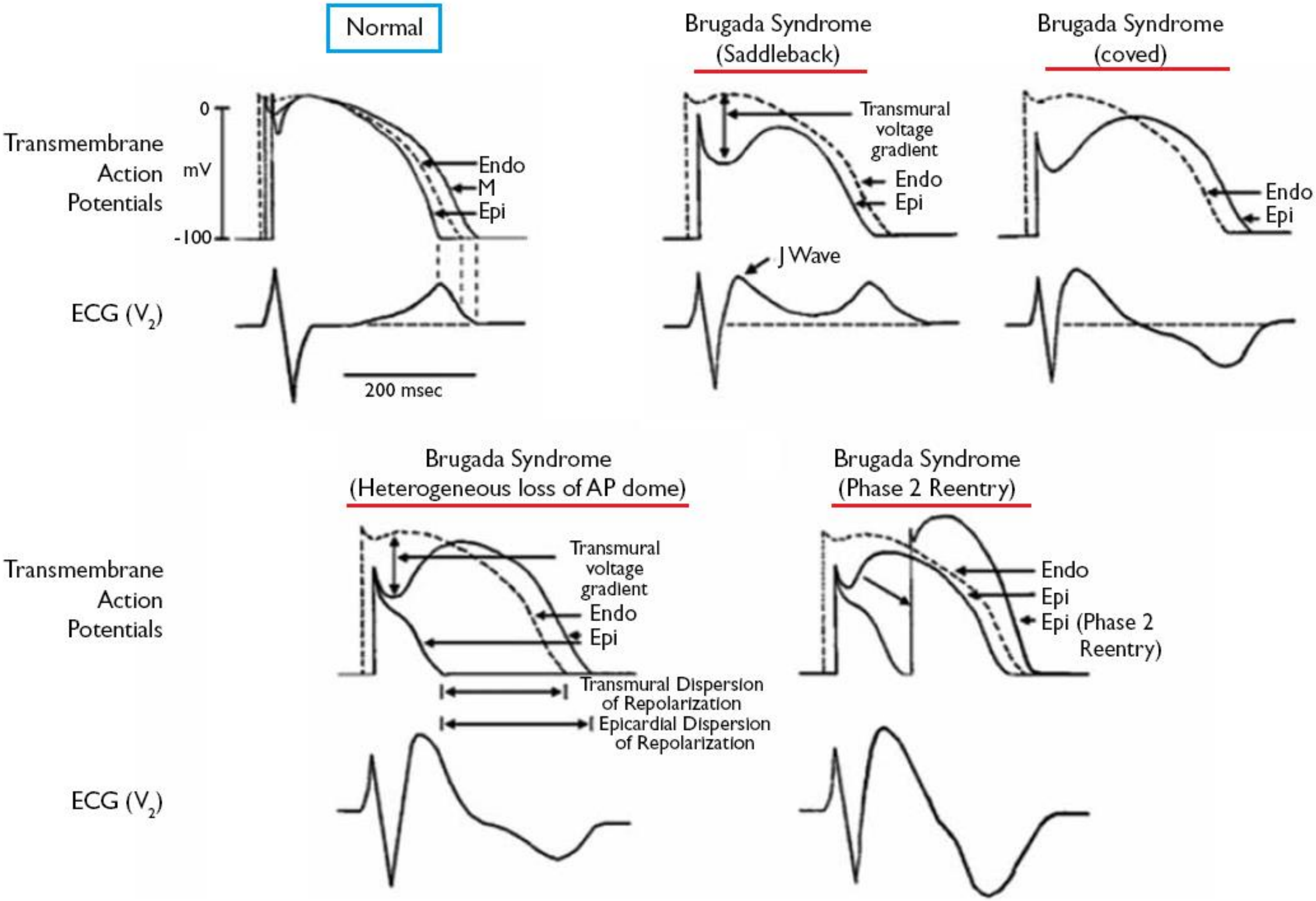




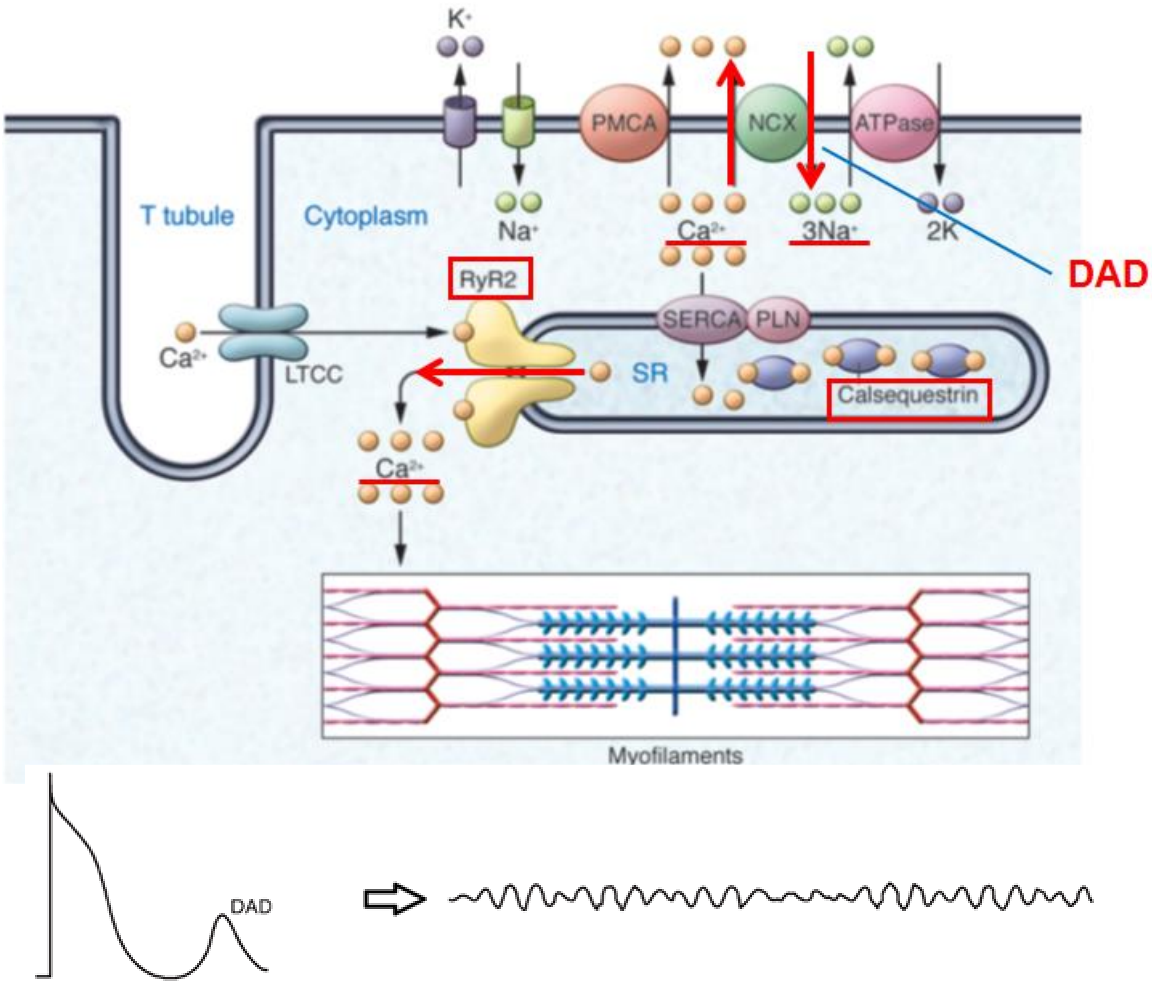
Short QT syndrome (SQTS)



Brugada syndrome



Catecholaminergic polymorphic ventricular tachycardia (CPVT)



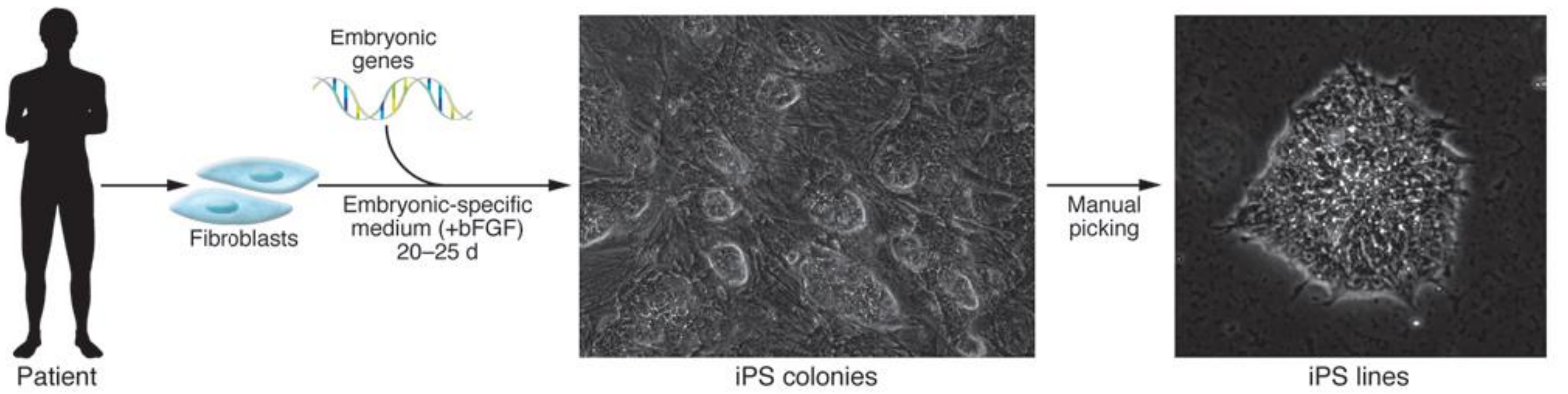
Genes involved in inherited arrhythmias

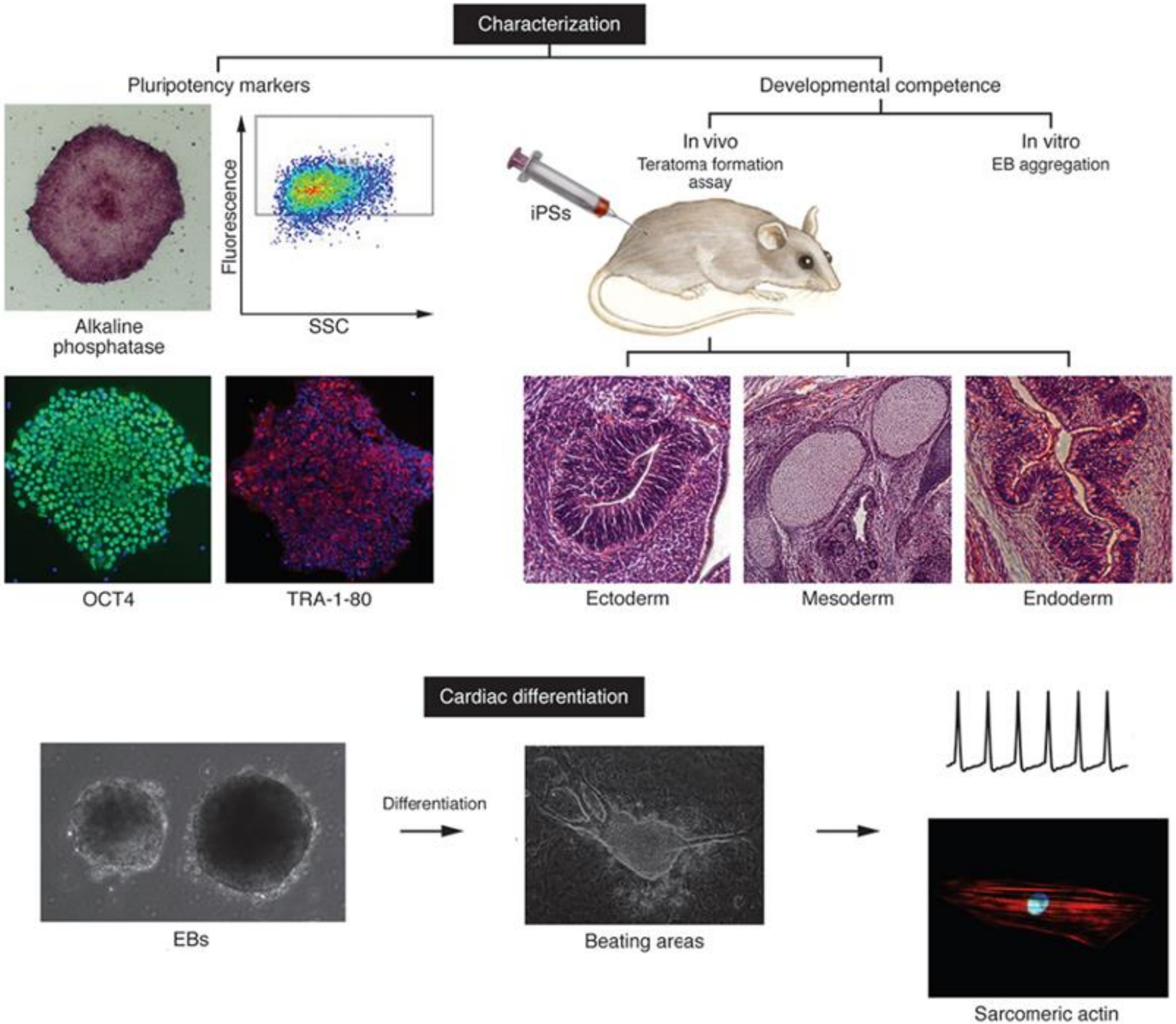
Phenotype	Gene[A]	Protein	Effect of mutation	OMIM identifier[B]
LQTS	*KCNQ1* (11p15.5)	K^+ voltage-gated channel, KQT-like subfamily, member 1 ($K_V7.1$)	Loss of function, reduced I_{Ks}	607542
	KCNH2 (7q35)	K^+ voltage-gated channel, subfamily H (eag-related), member 2 ($K_V11.1$; HERG)	Loss of function, reduced I_{Kr}	152427
	SCN5A (3p21)	Na^+ channel, voltage-gated, type V, α subunit ($Na_V1.5$)	Impaired inactivation, increased persistent I_{Na}	600163
	ANK2 (4q25)	Ankyrin 2, neuronal	Aberrant localization of ion transporters	106410
	KCNE1 (21q22.1)	K^+ voltage-gated channel auxiliary subunit	Reduced I_{Ks}	176261
	KCNE2 (21q22.1)	K^+ voltage-gated channel auxiliary subunit	Reduced I_{Kr}	603796
	CAV3 (3p25)	Caveolin 3	Increased persistent I_{Na}	601253
	SCN4B (11q23)	Na^+ channel, voltage-gated, type IV, β subunit	Increased persistent I_{Na}	608256
	SNTA1 (20q11.2)	Syntrophin, $\alpha 1$	Increased persistent I_{Na}	601017
	AKAP9 (7q21)	A kinase (PRKA) anchor protein (*yotiao*) 9	Reduced I_{Ks}	604001
	KCNJ5 (11q24)	K^+ inwardly rectifying channel, subfamily J, member 5 (Kir3.4)	Reduced $I_{K,ACh}$	600734
Jervell and Lange-Nielson syndrome	*KCNQ1* (11p15.5)	K^+ voltage-gated channel, KQT-like subfamily, member 1 ($K_V7.1$)	Loss of function, reduced I_{Ks}	607542
	KCNE1 (21q22.1)	K^+ voltage-gated channel auxiliary subunit	Reduced I_{Ks}	176261
Andersen syndrome	*KCNJ2* (17q23.1)	K^+ inwardly rectifying channel, subfamily J, member 2 (Kir2.1)	Loss of function, reduced I_{K1}	600681
Timothy syndrome	*CACNA1C* (12p13.3)	Ca^{2+} channel, voltage-dependent, L type, α1C subunit ($Ca_V1.2$)	Gain of function, increased I_{Ca}	114205

Genes involved in inherited arrhythmias

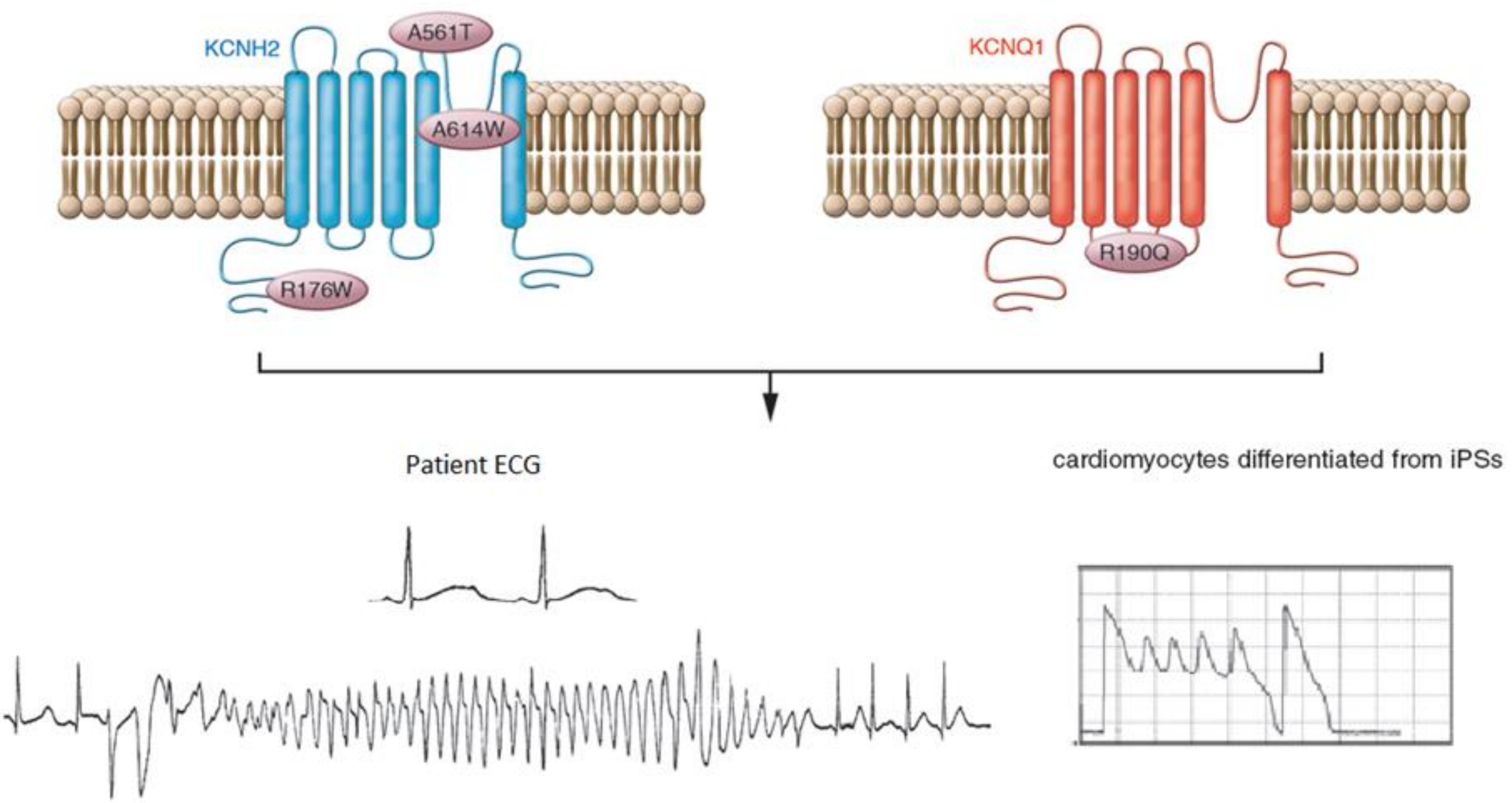
Phenotype	Gene[A]	Protein	Effect of mutation	OMIM identifier[B]
SQTS	*KCNQ1* (11p15.5)	K^+ voltage-gated channel, KQT-like subfamily, member 1 (K_V7.1)	Gain of function, increased I_{Ks}	607542
	KCNH2 (7q35)	K^+ voltage-gated channel, subfamily H (eag-related),member 2 (K_V11.1; HERG)	Gain of function, increased I_{Kr}	152427
	KCNJ2 (17q23.1)	K^+ inwardly rectifying channel, subfamily J, member 2 (Kir2.1)	Gain of function, increased I_{K1}	600681
	CACNA1C (12p13.3)	voltage-gated Ca^{2+} channel, Ca_V1.2	Loss of function, reduced I_{Ca}	114205
	CACNB2 (10p12)	Ca^{2+} channel, voltage-dependent, β2 subunit	Loss of function, reduced I_{Ca}	600003
	CACNA2D1 (7q21)	Ca^{2+} channel, voltage-dependent, α2/δ subunit 1	Loss of function, reduced I_{Ca}	114204
BrS	*SCN5A* (3p21)	Na^+ channel, voltage-gated, type V, α subunit (Na_V1.5)	Loss of function, reduced I_{Na}	600163
	GPD1L (3q22.3)	glycerol-3-phosphate dehydrogenase 1-like	Reduced I_{Na}	611778
	SCN1B (19q13.1)	Na^+ channel, voltage-gated, type I, β subunit	Reduced I_{Na}	600235
	SCN3B (11q23.3)	Na^+ channel, voltage-gated, type III, β subunit	Reduced I_{Na}	608214
	MOG1 (17p13.1)	RAN guanine nucleotide release factor	Reduced I_{Na}	607954
	KCND3 (1p13.3)	K^+ voltage-gated channel, Shal-related subfamily, member 3 (K_V4.3)	Gain of function, increased I_{to}	605411
	KCNE3 (11q13)	K^+ voltage-gated channel auxiliary subunit	Increased I_{to}	604433
	KCNE5 (Xq22.3)	K^+ voltage-gated channel auxiliary subunit	Increased I_{to}	300328
	CACNA1C (12p13.3)	Ca^{2+} channel, voltage-dependent, L type, α1C subunit (Ca_V1.2)	Loss of function, reduced I_{Ca}	114205
	CACNB2 (10p12)	Ca^{2+} channel, voltage-dependent, β2 subunit	Loss of function, reduced I_{Ca}	60003
	KCNJ8 (12p12.1)	K^+ inwardly rectifying channel, subfamily J, member 8 (Kir6.1)	Gain of function, increased $I_{K,ATP}$	600935
CPVT	*RYR2* (1q42.1)	Ryanodine receptor 2 cardiac	Gain of function, increased SR Ca^{2+} release	180902
	CASQ2 (1p13.3)	Calsequestrin 2 cardiac muscle	Loss of function, reduced I_{Ca}	114251
	TRDN (6q22)	Triadin	Impaired regulation of SR Ca^{2+} release	603283

Experiment of inherited arrhythmias with induced pluripotent stem cells (iPSs)

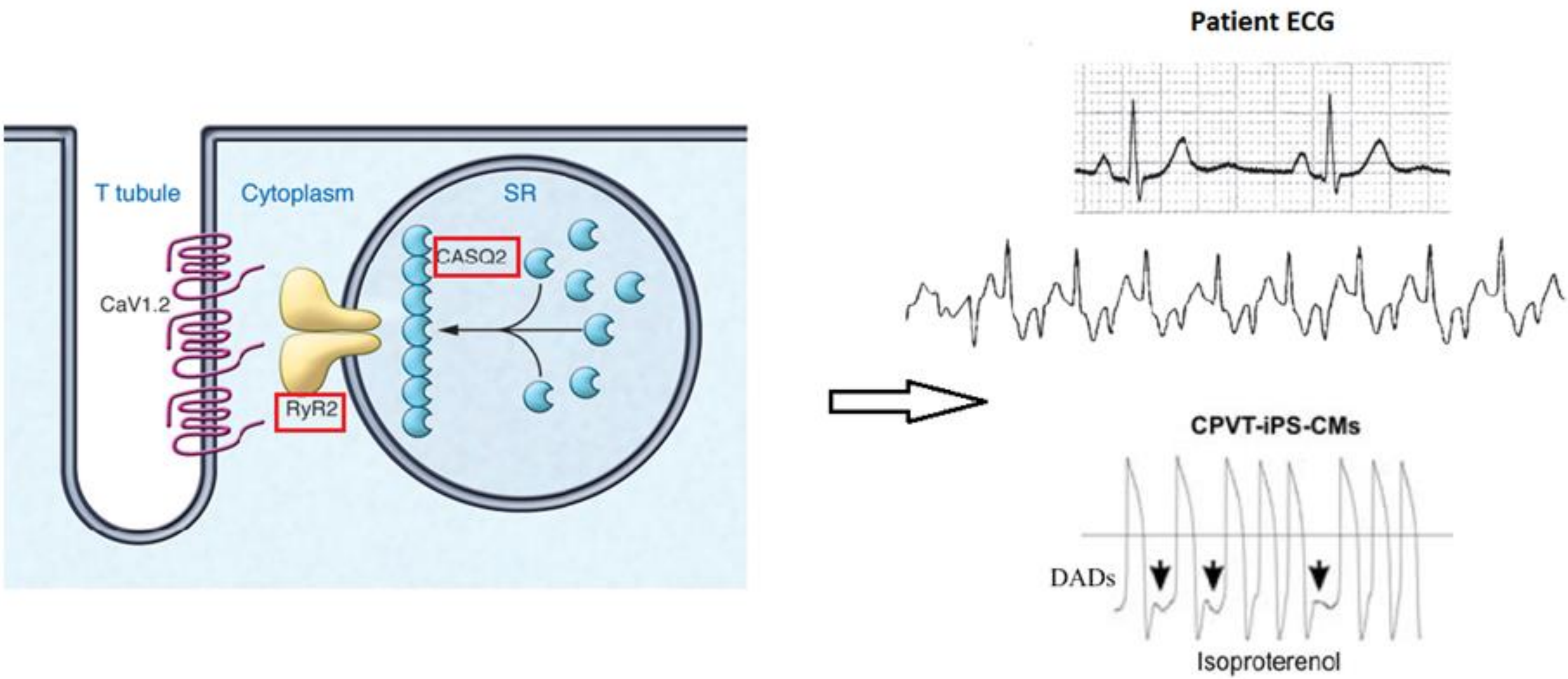



Characterization
Pluripotency markers
Developmental competence
Fluorescence
SSC
Alkaline phosphatase
In vivo
Teratoma formation assay
In vitro
EB aggregation
iPSs
OCT4
TRA-1-80
Ectoderm
Mesoderm
Endoderm
Cardiac differentiation
Differentiation
EBs
Beating areas
Sarcomeric actin

Modeling LQTS with iPSs



Modeling CPVT with iPSs



Summary of studies with iPSs

Summary of studies of iPS-derived cardiomyocytes from patients with inherited arrhythmias

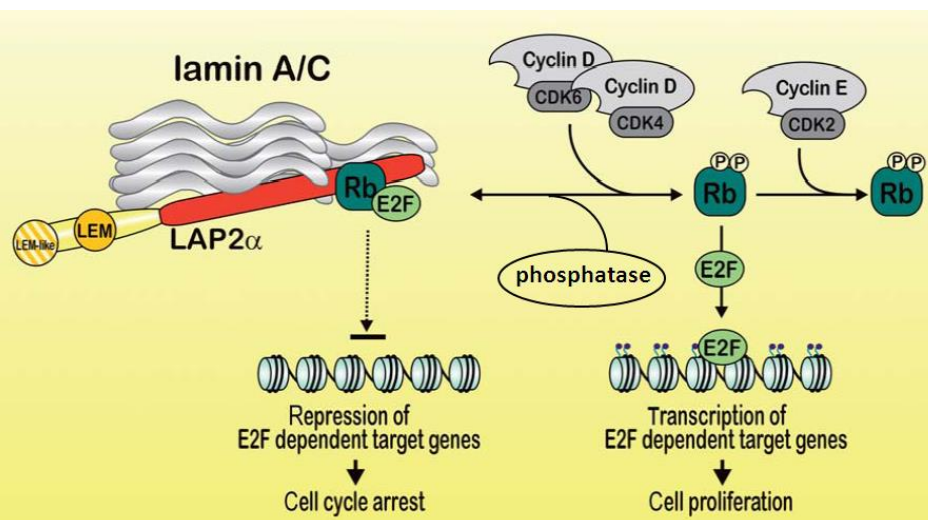
Phenotype	*N*	AP frequency (Hz)	MDP (mV)	APA (mV)	Maximal upstroke velocity (dV/dt)	APD50 (ms)	APD90 (ms)	Age (d)	Syndrome	Reference
Control cells										
Working	31	1.43 ± 0.11	–58 ± 1.6	97 ± 2.7	44 ± 6.7	145 ± 16	211 ± 17	20–25		27
Ventricular	32	1.70 ± 0.10	–76 ± 1.2	104 ± 1.1	28 ± 4.8		414 ± 22	30–32		46
Ventricular	37	0.73 ± 0.04	–63 ± 1.7	88 ± 2.6		241 ± 15	320 ± 17	10/20/30		25
Ventricular	39	0.73 ± 0.05	–63 ± 1.5	88 ± 2.4		239 ± 10	312 ± 11.20	10/20/30		25
Ventricular	40	1.00[A]	–66 ± 1.2	108 ± 1.2		318 ± 19	373 ± 22	30–90	LQT1	31
Ventricular	NA					221 ± 85[B]	297 ± 118[B]	25–30	LQT2	35
Ventricular	60	0.46 ± 0.10	–57 ± 1.0	109 ± 3	9.5 ± 1.8	308 ± 24[B]	436 ± 23[B]		LQT2	33
Ventricular	13	1.2 ± 0.10	–63 ± 1.3	113 ± 2.4		265 ± 15	311 ± 20		LQT2	34
Ventricular	16						400 ± 45[B]	37	LQT8	36
Ventricular	15							25	AD-CPVT	40
Ventricular	9	1.00	–75 ± 3.0			252 ± 29[B]		30–120	AD-CPVT	41
Working	10	0.64 ± 0.06	–58 ± 3.0[B]	99 ± 3[B]	6.20 ± 0.1[B]	201[B] ± 27[B]		21	AR-CPVT	43
Patient-derived cells										
Ventricular	36	1.00[A]	–67 ± 1.20	110 ± 1.3		481 ± 33	554 ± 35	30–90	LQT1	31
Ventricular	NA					454 ± 90[B]	635 ± 119[B]	25–30	LQT2	35
Ventricular	58	0.26 ± 0.30	–55 ± 2	116 ± 4	10 ± 1.3	440 ± 9[B]	864 ± 8[B]		LQT2	33
Ventricular	13	0.90 ± 0.10	–62 ± 0.90	117 ± 1.4		455 ± 26	516 ± 26	180	LQT2	34
Working	16						1,130 ± 150[B]	37	LQT8	36
Ventricular	16	1.00[A]	79 ± 2.70[B]			234 ± 21[B]	293 ± 23[B]	60–120	AD-CPVT	41
Working	20		–56 ± 1[B]	98 ± 1.0[B]	7.60 ± 1.2[B]	368 ± 41[B]		21	AD-CPVT	43
Ventricular	24							25	AR-CPVT	40

[A]Experiments with electrically stimulated cells (nonspontaneous beating). [B]Data derived from graphs. AD, autosomal dominant; AR, autosomal recessive; AP, action potential; MDP, maximum diastolic potential; APA, action potential amplitude.

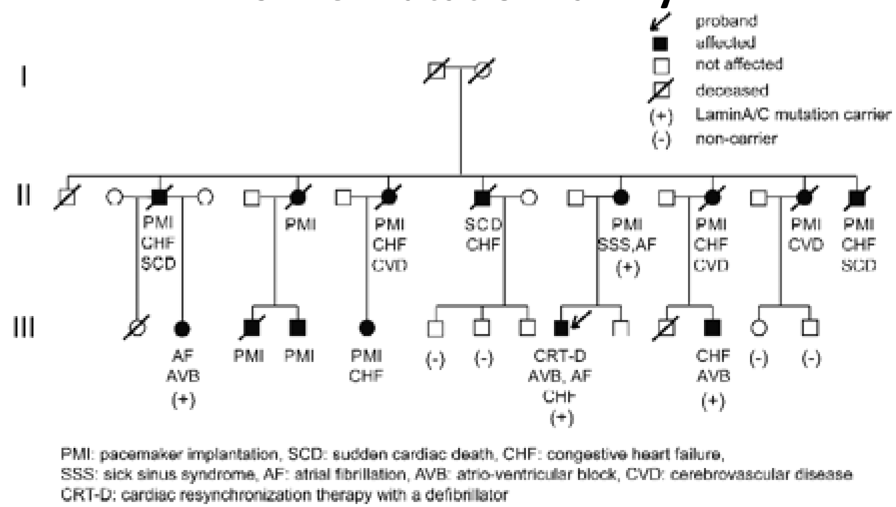
R644C Mutation of Lamin A Causes Cardiac Fibroblasts Senescence

Background

- Lamin A/C
  - Nuclear membrane protein
  - It affects cell proliferation
  - Mutation → cardiomyopathy, arrhythmia, progeria

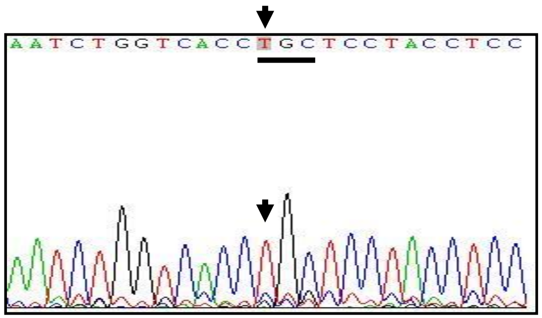


R644C mutation family



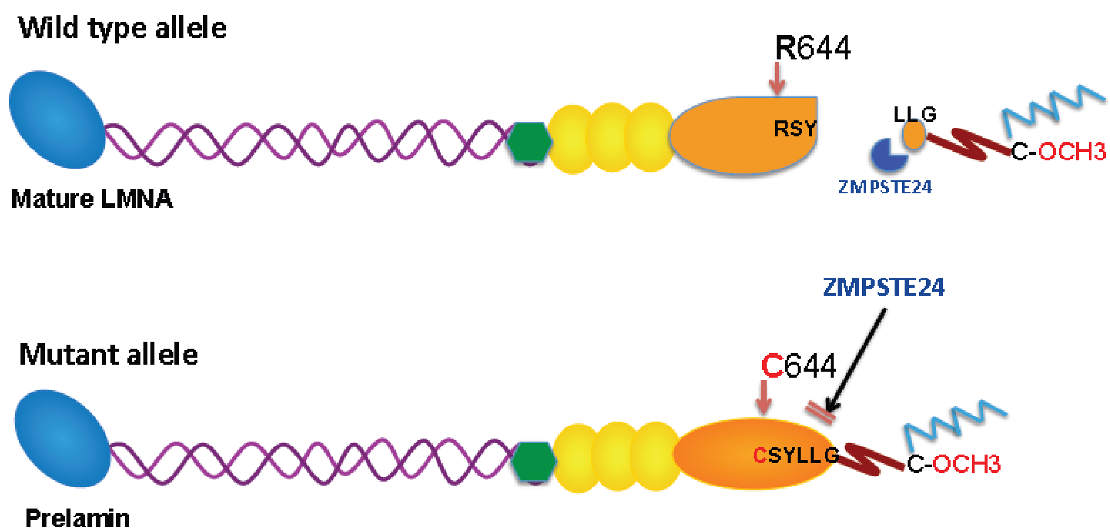
Lamin A, R644C mutation

R644C (24050 C>T)



R644C (Arg>Cys)

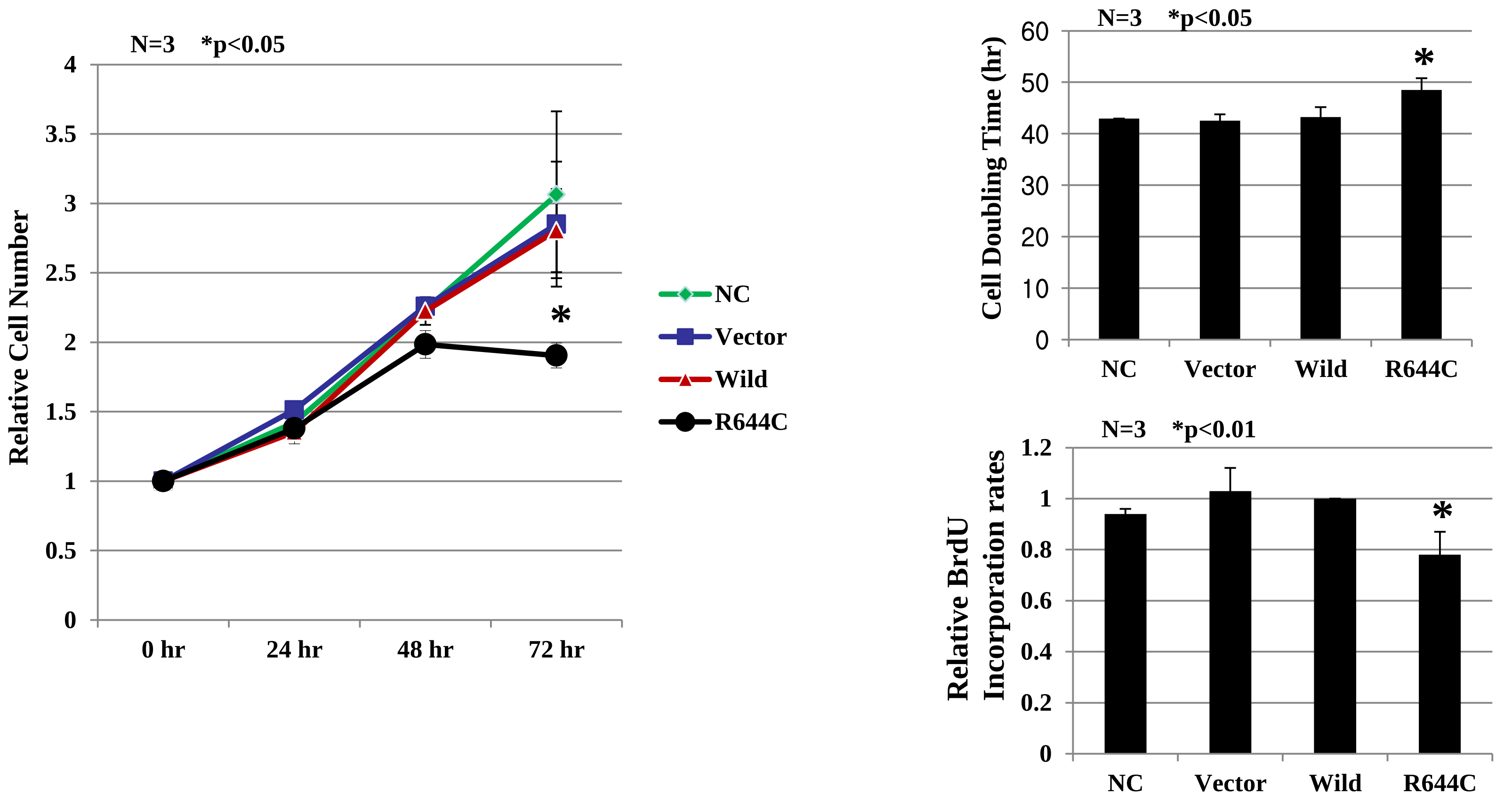
Homo sapiens	DNLVTRSYLLGN	650
Pan troglodytes	DNLVTRSYLLGT	650
Macaca mulatta	DNLVTRSYLLGN	712
Sus scrofa	DNLVTRSYLLGN	650
Canis familiaris	DNLVTRSYLLGS	651
Mus muluscus	DNLVTRSYLLGN	651



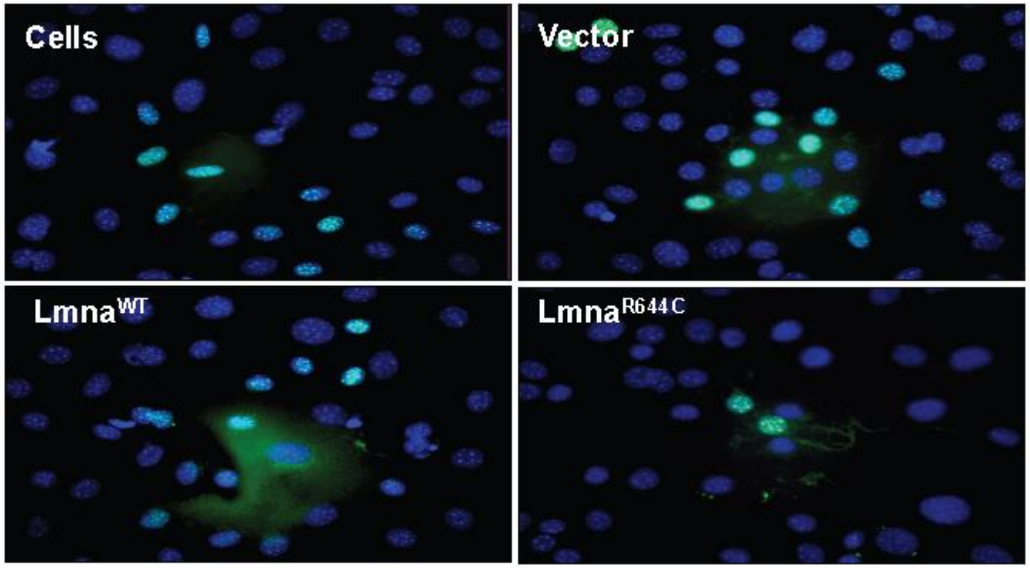
Methods

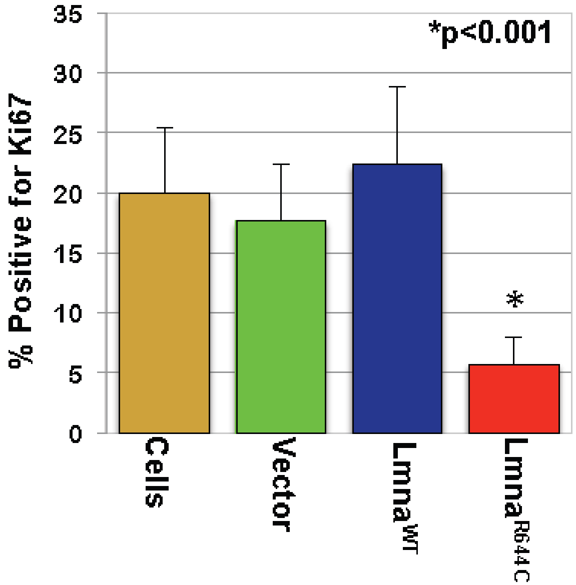
We generated recombinant adenoviruses and expressed Flag-tagged wild type Lmna (LmnaWT) and mutant Lmna R644C in fibroblasts isolated from mouse hearts.

Lamin A Mutation (R644C) decreases Cell Proliferation In Mouse Cardiac Fibroblasts

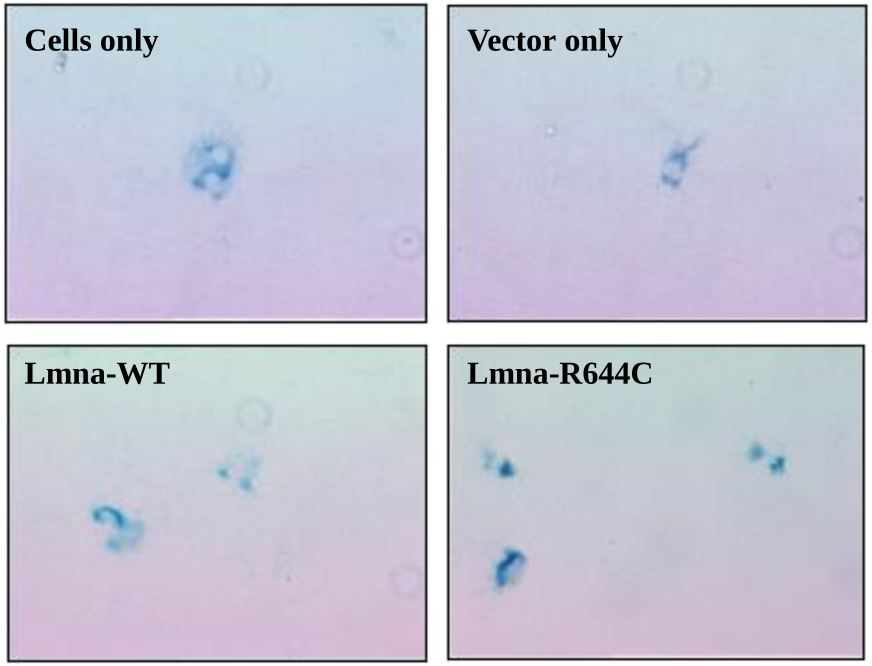


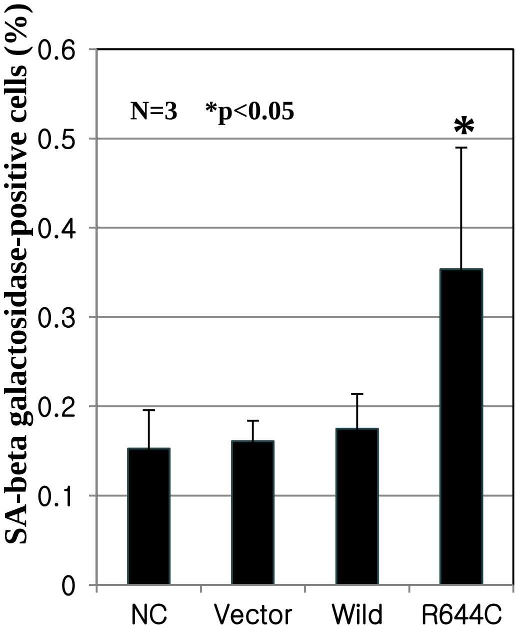
Cell Proliferation (Ki67 expression) is reduced in Lamin A Mutation (R644C) In Mouse Cardiac Fibroblasts



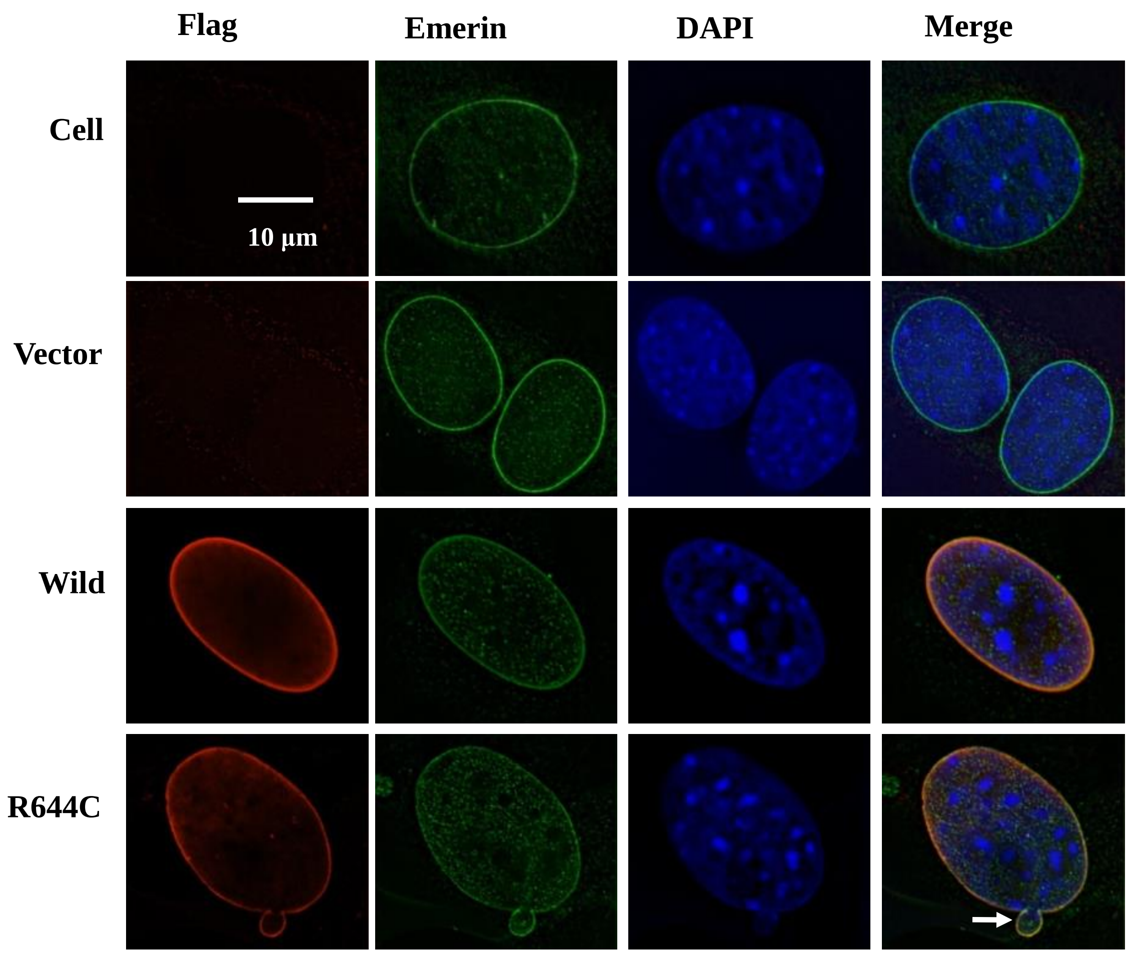


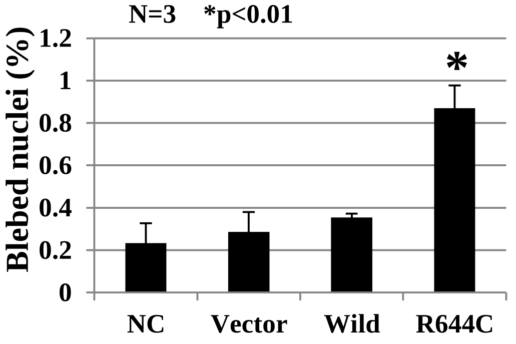
Lamin A Mutation (R644C) causes Cellular Senescence In Mouse Cardiac Fibroblasts
(SA-beta galactosidase assay)



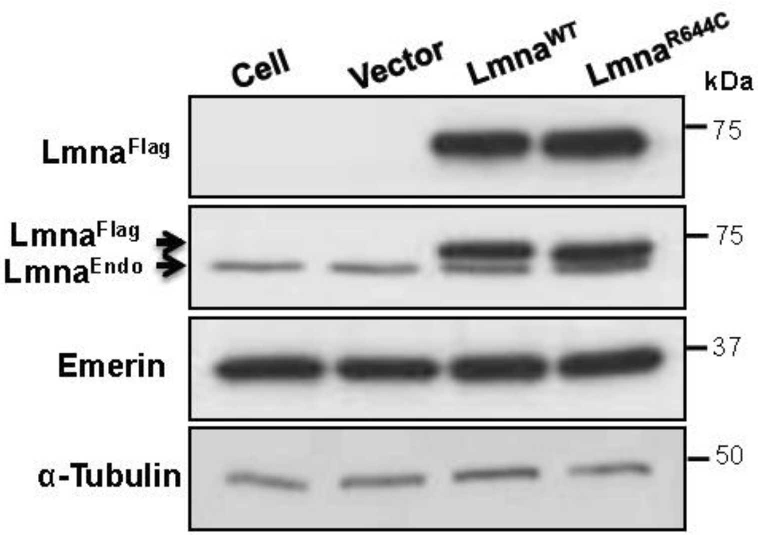


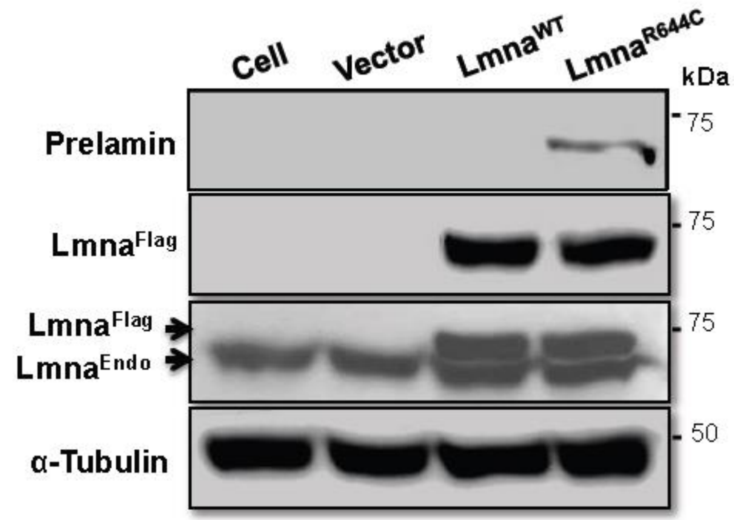
Lamin A Mutation (R644C) causes Nucleus Blebbing In Mouse Cardiac Fibroblasts



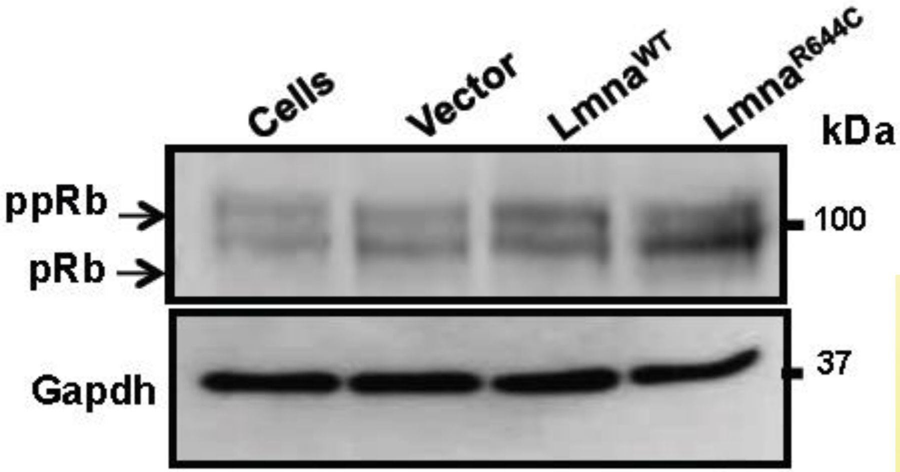


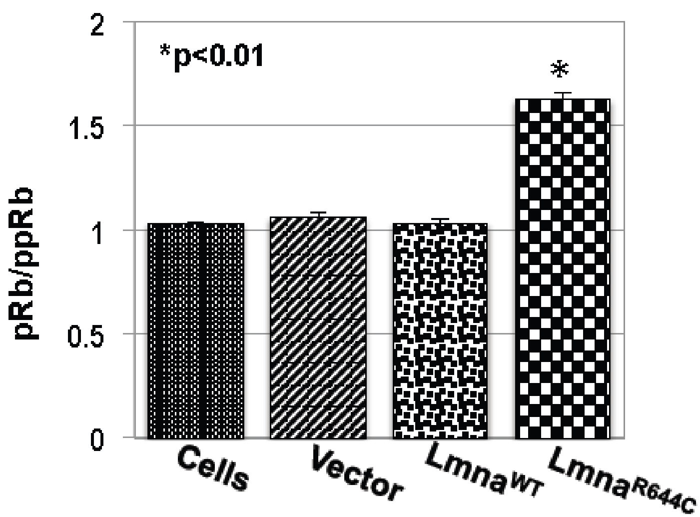
Lamin A Mutation (R644C) causes prelamin expression In Mouse Cardiac Fibroblasts

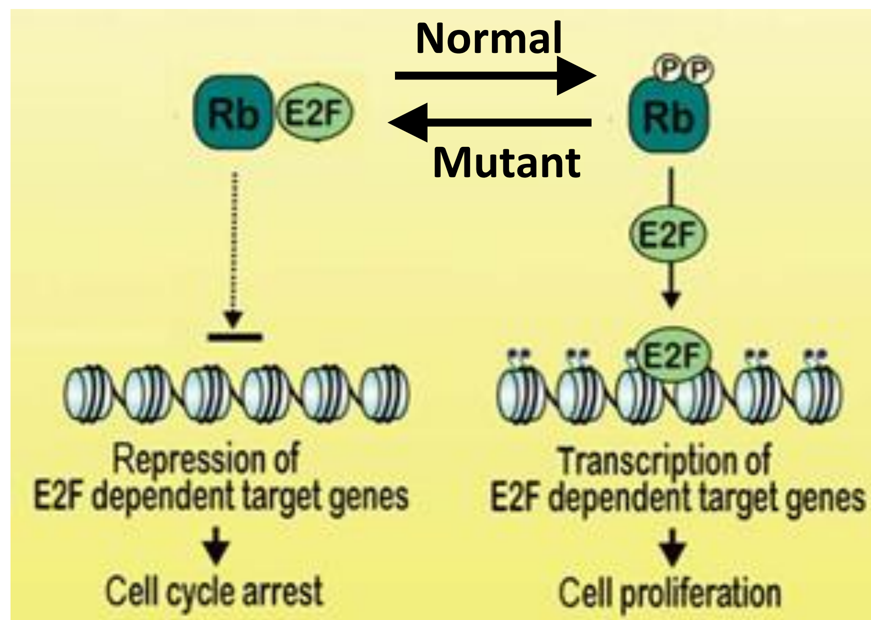




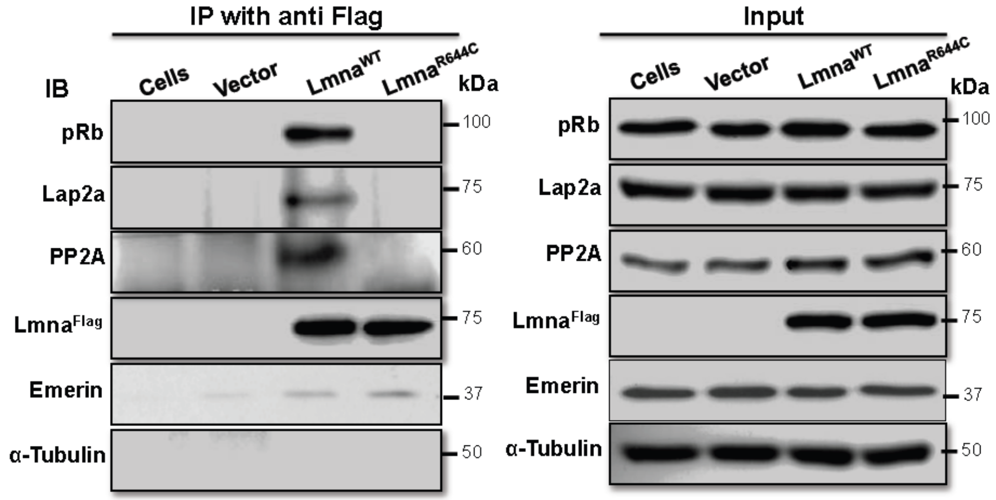
Lamin A Mutation (R644C) reduces Rb phosphorylation In Mouse Cardiac Fibroblasts

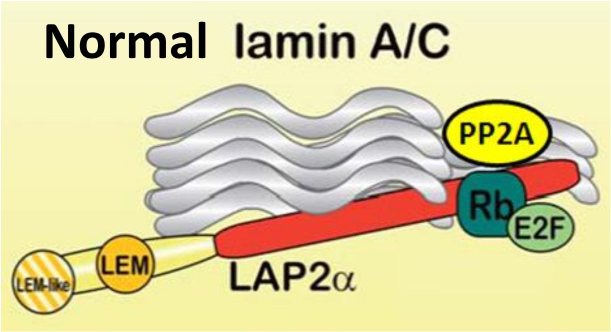


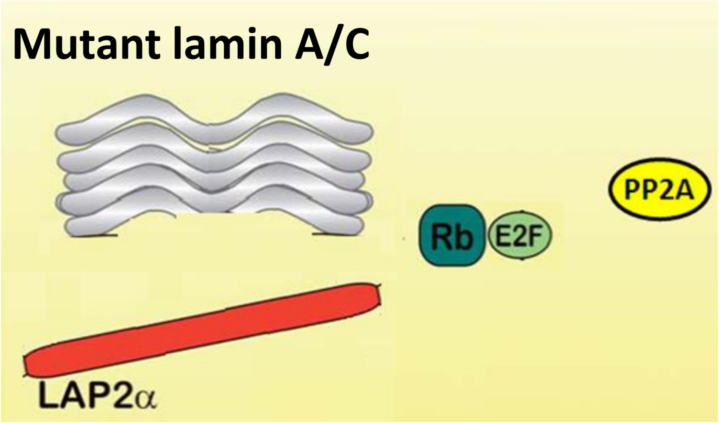




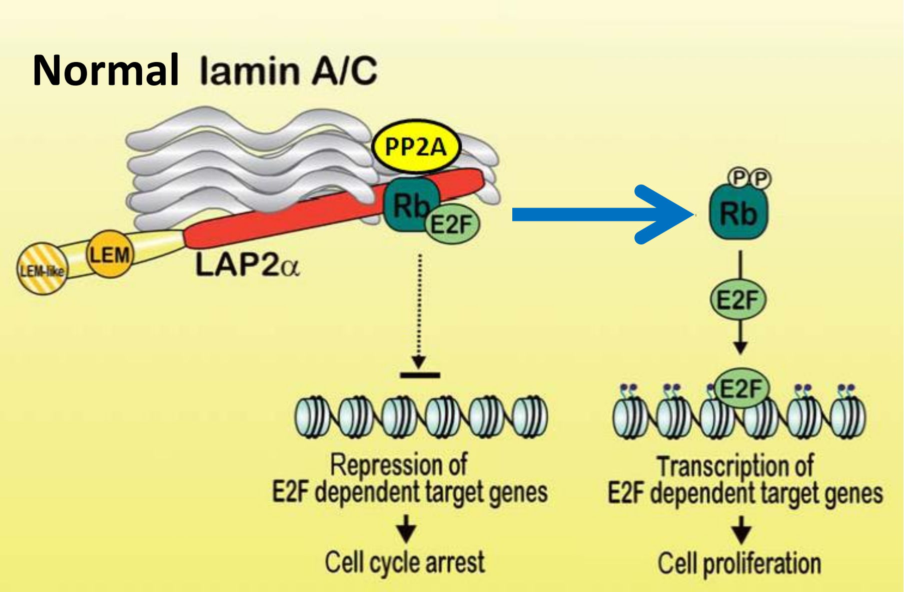
Lamin A Mutation (R644C) causes dissociation of binding proteins in Mouse Cardiac Fibroblasts

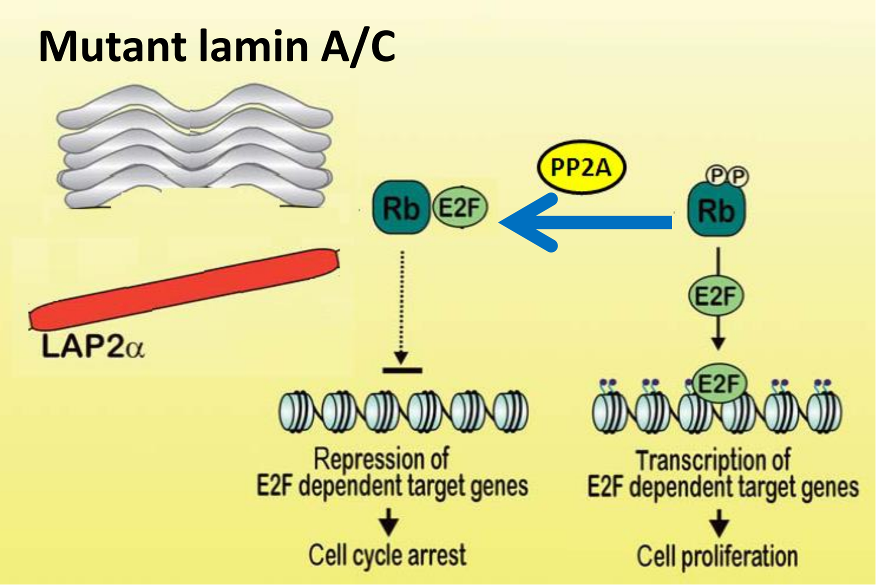






Hypothesis

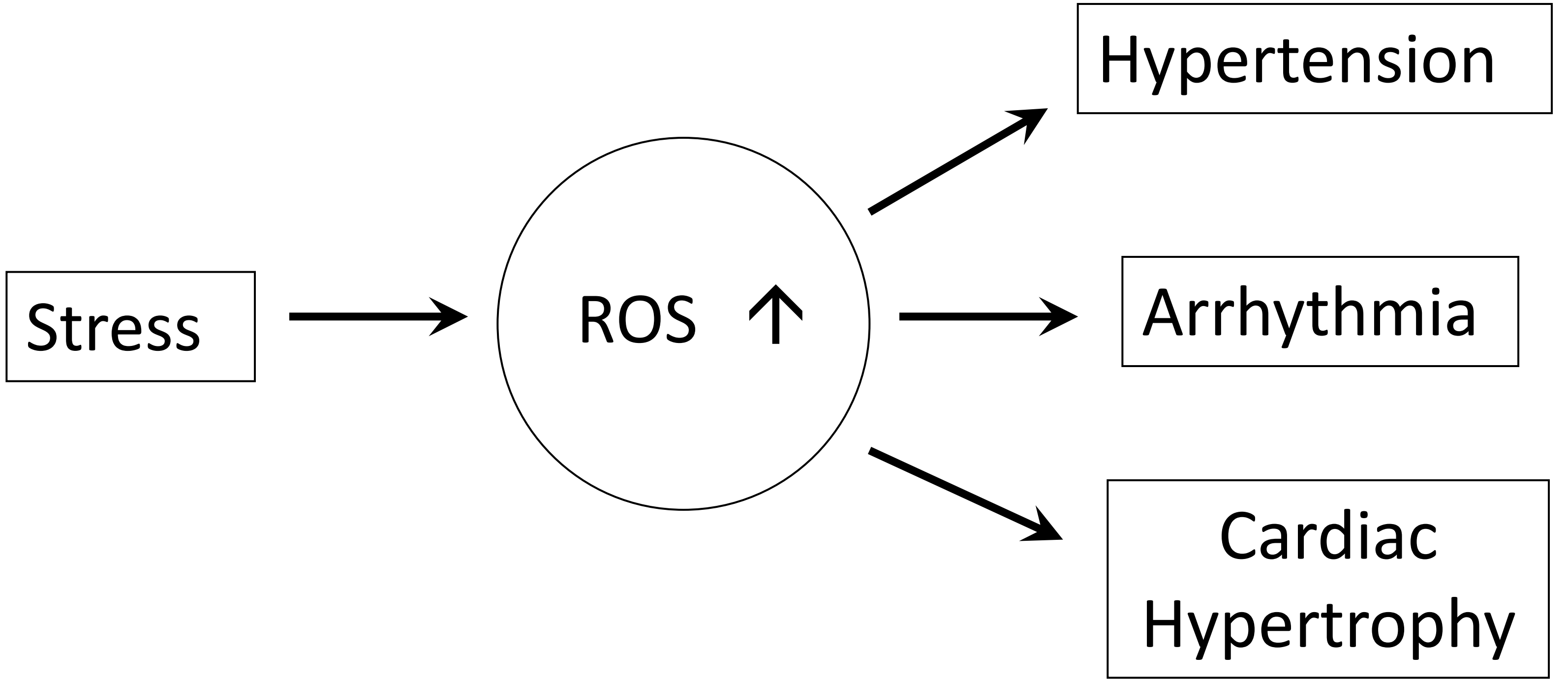

Normal lamin A/C
PP2A
Rb
E2F
LEM-like
LEM
LAP2α
P P
Rb
E2F
E2F
Repression of
E2F dependent target genes
Cell cycle arrest
Transcription of
E2F dependent target genes
Cell proliferation


Mutant lamin A/C
PP2A
Rb
E2F
P P
Rb
LAP2α
E2F
E2F
Repression of
E2F dependent target genes
Cell cycle arrest
Transcription of
E2F dependent target genes
Cell proliferation

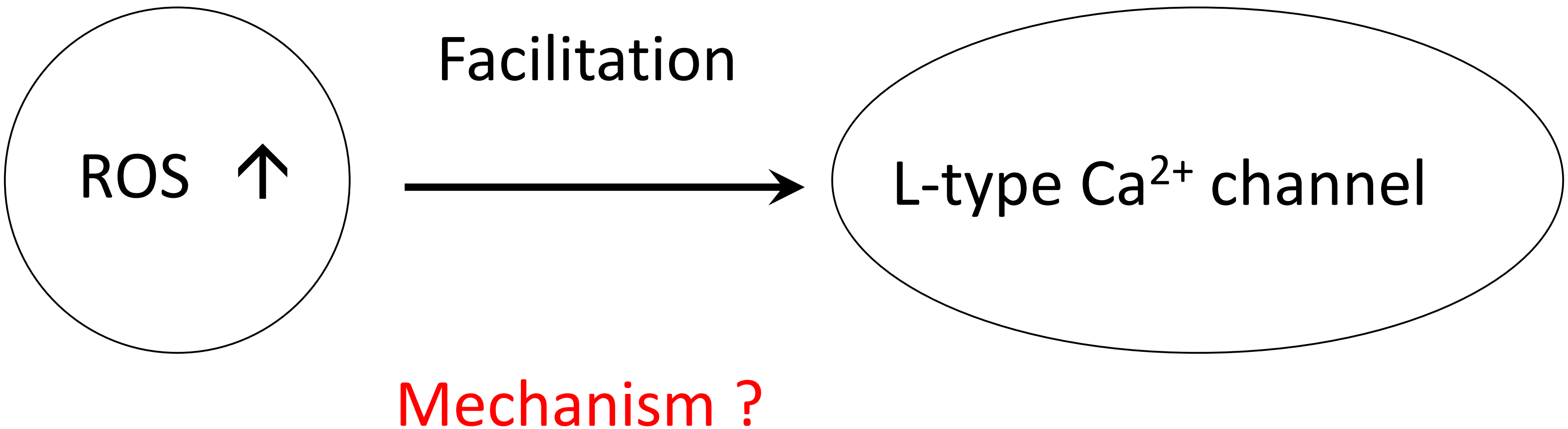
L-type Ca^{2+} channel activation by ROS-induced activation of CaMKII.

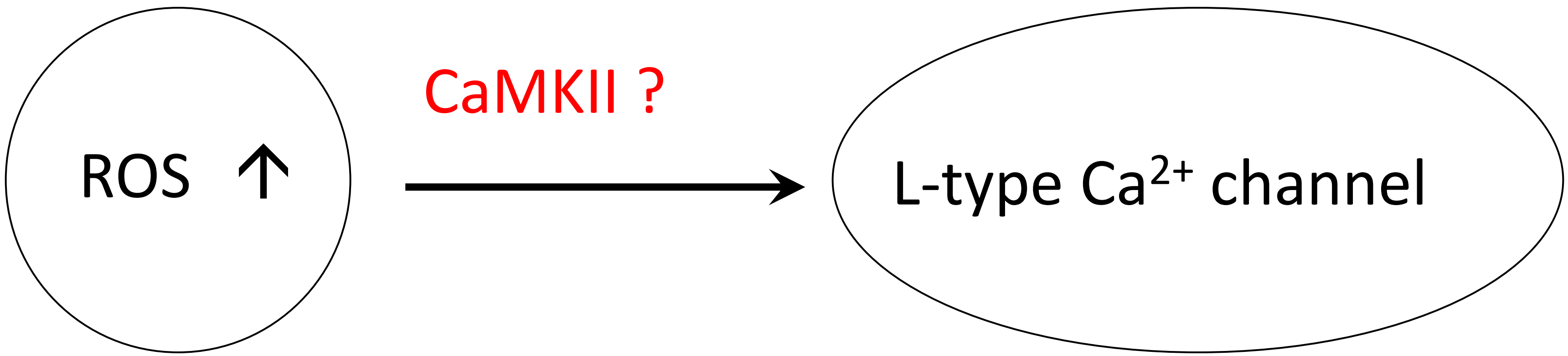
Introduction

Cardiovascular System

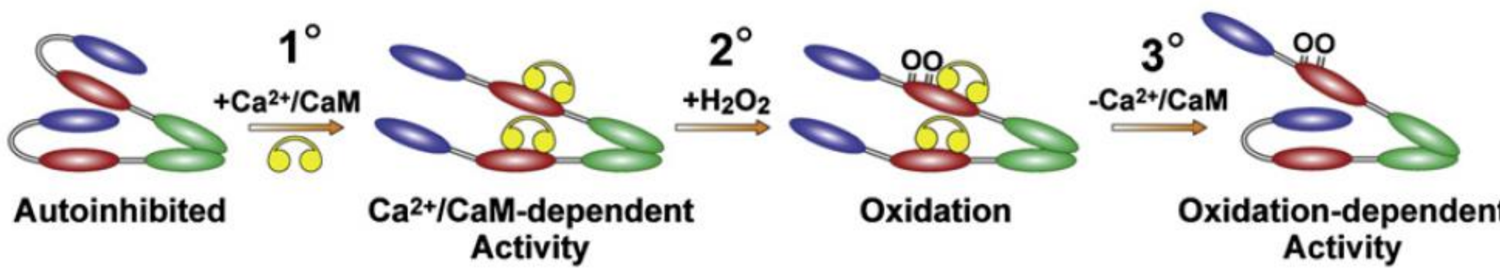


Cardiac Myocyte





1) ROS → CaMKII



2) CDF: CaMKII mediated reaction

Study Plan

- Effect of ROS on L-type Ca^{2+} channel
- CaMKII: Involvement & mechanism
- Role of Ca^{2+}
- Comparison with CDF
- Long term potentiation: LTP

Materials & Methods

Chemicals and Solutions

- Normal Tyrode solution (mM): 140 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1.8 $CaCl_2$, 10 glucose, and 5 HEPES, titrated to pH 7.4 with NaOH.
- Ca^{2+}-free solution (mM): 140 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 10 glucose, and 5 HEPES, titrated to pH 7.4 with NaOH.
- The high K^+, low Cl^- solution (mM): 70 KOH, 40 KCl, 50 L-glutamic acid, 20 taurine, 20 KH2PO4, 3 MgCl2, 10 glucose, 10 HEPES, and 0.5 EGTA.
- The pipette solution (mM): 100 CsOH, 110 gluconic acid, 10 NaCl, 20 HEPES, 20 tetraethylammonium-Cl, 4 Mg-ATP, 5 Na-phosphocreatine, and 10 EGTA titrated to pH 7.3 with CsOH.
- Drugs were prepared as concentrated stock solutions either in distilled water or dimethyl sulfoxide.
- All experiments were conducted at room temperature (22-25℃).

Cell Isolation

- Male Sprgue-Dawley rat
- The removed heart was perfused with digestion solution containing collagenase through Langendorff system.
- The ventricles were cut into small pieces and individual myocytes were obtained by gentle trituration.
- The isolated cells were stored in the high K^+, low Cl^- solution at 4℃ until used in experiments.

Voltage Clamp Recording & Analysis

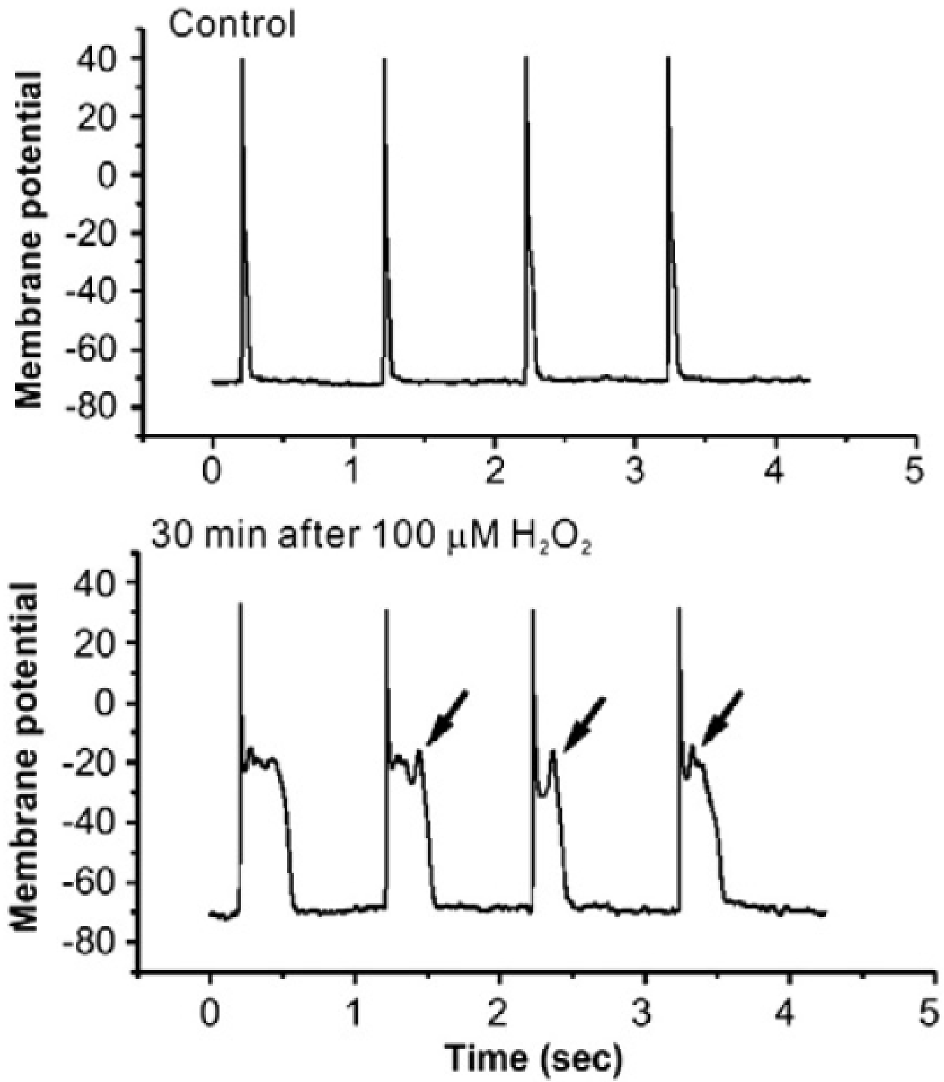
- Patch pipettes were pulled from borosilicate glass capillaries (Clark Electromedical Instruments, UK) using a pipette puller (model PP-83, Narishige Scientific Instrument Lab. Janpan) and were fire polished.
- Pipettes exhibited 3 to 4 MΩ resistance when filled with a pipette solution
- Voltage clamp was performed by using the conventional whole cell method.
- All recordings were initiated at least 10 min after rupture of membrane to allow complete dialysis of the cytoplasm.
- All the $I_{ca,L}$ recording were made at room temperature (22-25℃) using Axopatch amplifier (Axon Instruments, CA).
- Signals from the patch amplifier were filtered at 1 kHz and digitized with an A/D converter (PCI-6040E, National Instrument, USA) at a sampling rate of 1 kHz and stored on a hard disc installed in a personal computer using a software made in our laboratory (R-clamp, by SY Ryu) written with Delphi 6.0 (Borland Software Co.).

Western Blot

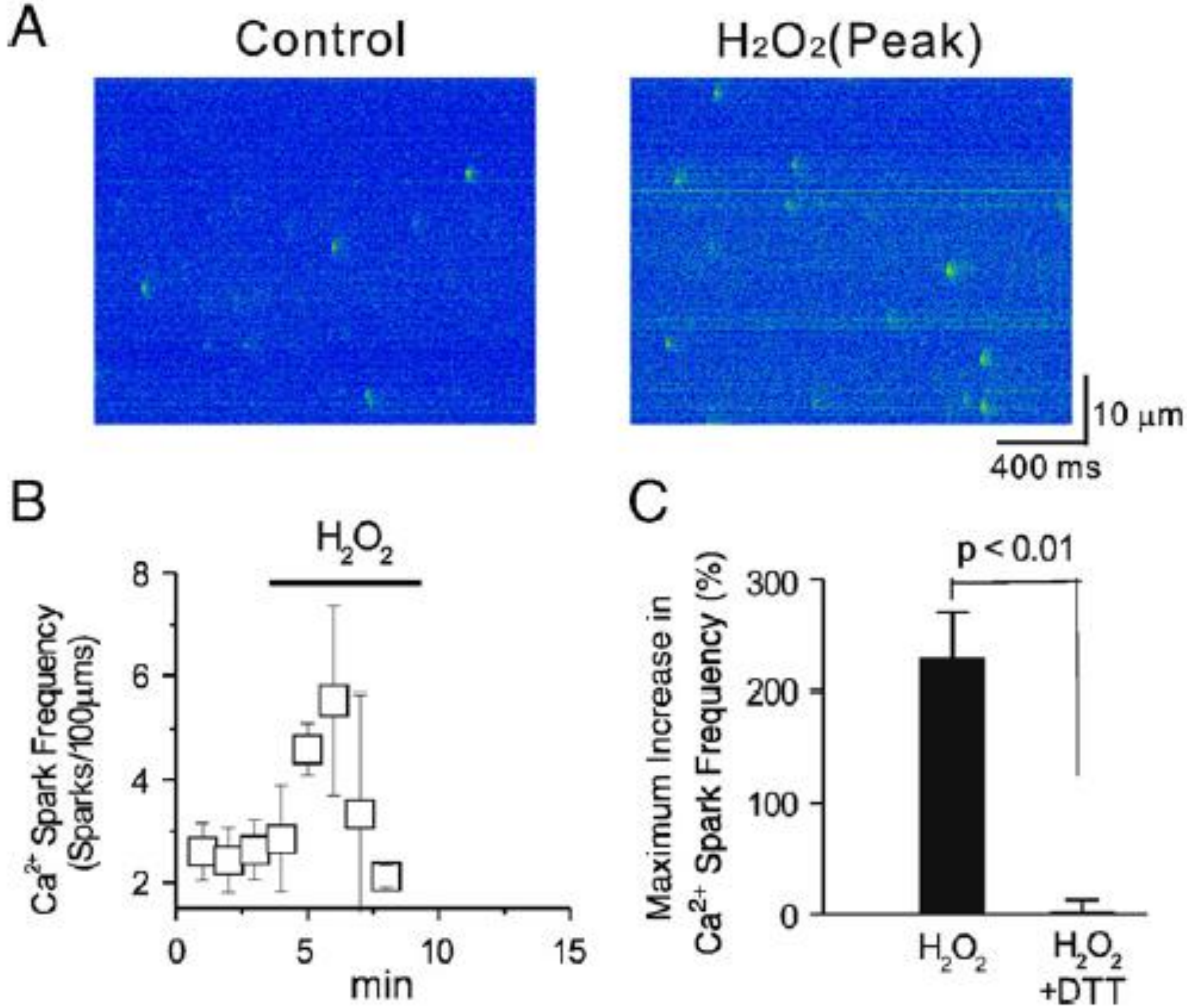
- The removed rat heart was perfused with specific solutions dependent on 4 each condition (+/- Ca^{2+}, +/- H_2O_2) through Langendorff system for 10 min.
- The ventricles were cut into small pieces and weighed about 300g and homogenized into the same solutions as perfused on previous step.
- the total proteins was quantified by Brad-Ford assay
- 100 g of protein from the each samples was separated on 10% SDS-polyacrylamide gel and electrophoretically transferred onto the polyvinylidene difluoride membrane

Results

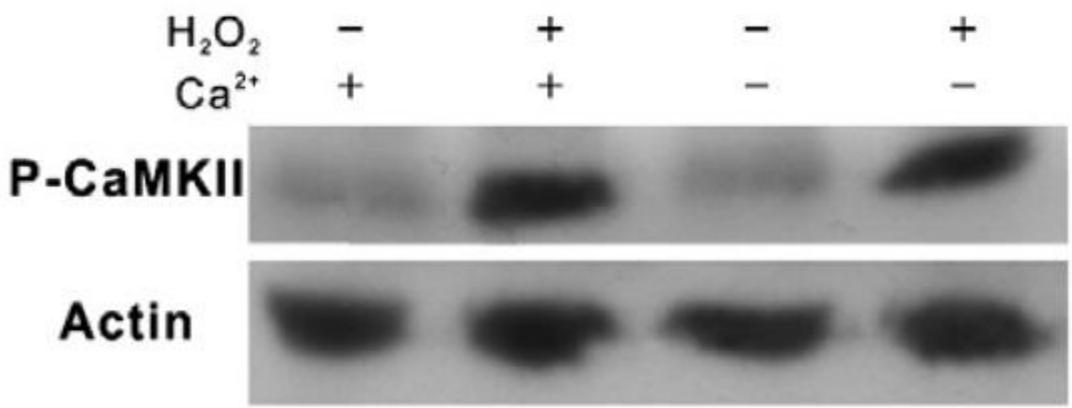
Action potential (AP) changes By H_2O_2



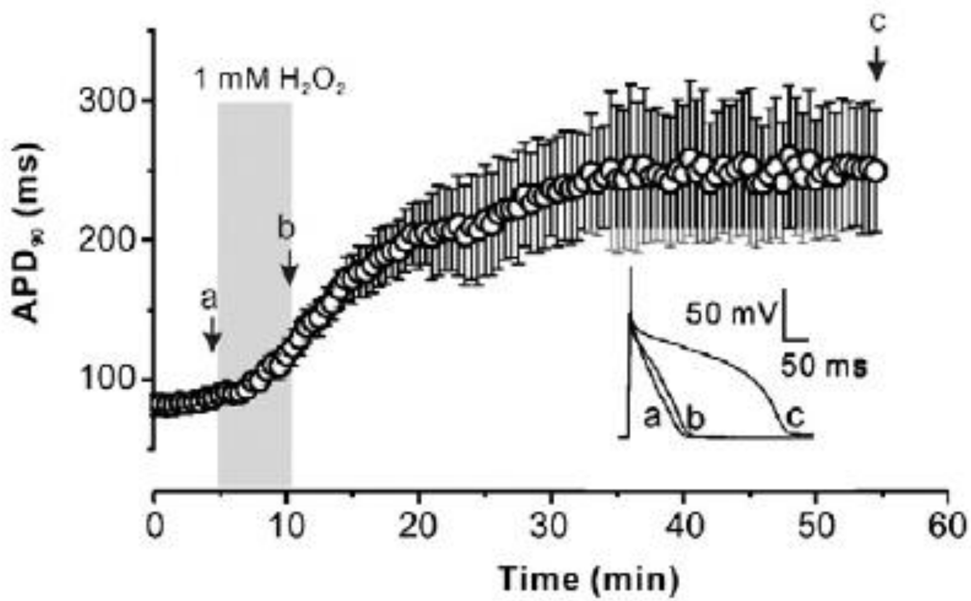
Spontaneous SR Ca^{2+} spark

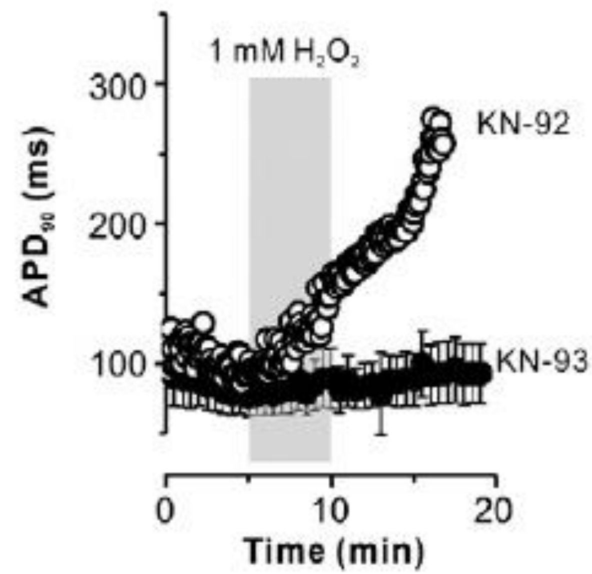


H_2O_2 increases phosphorylation of CaMKII

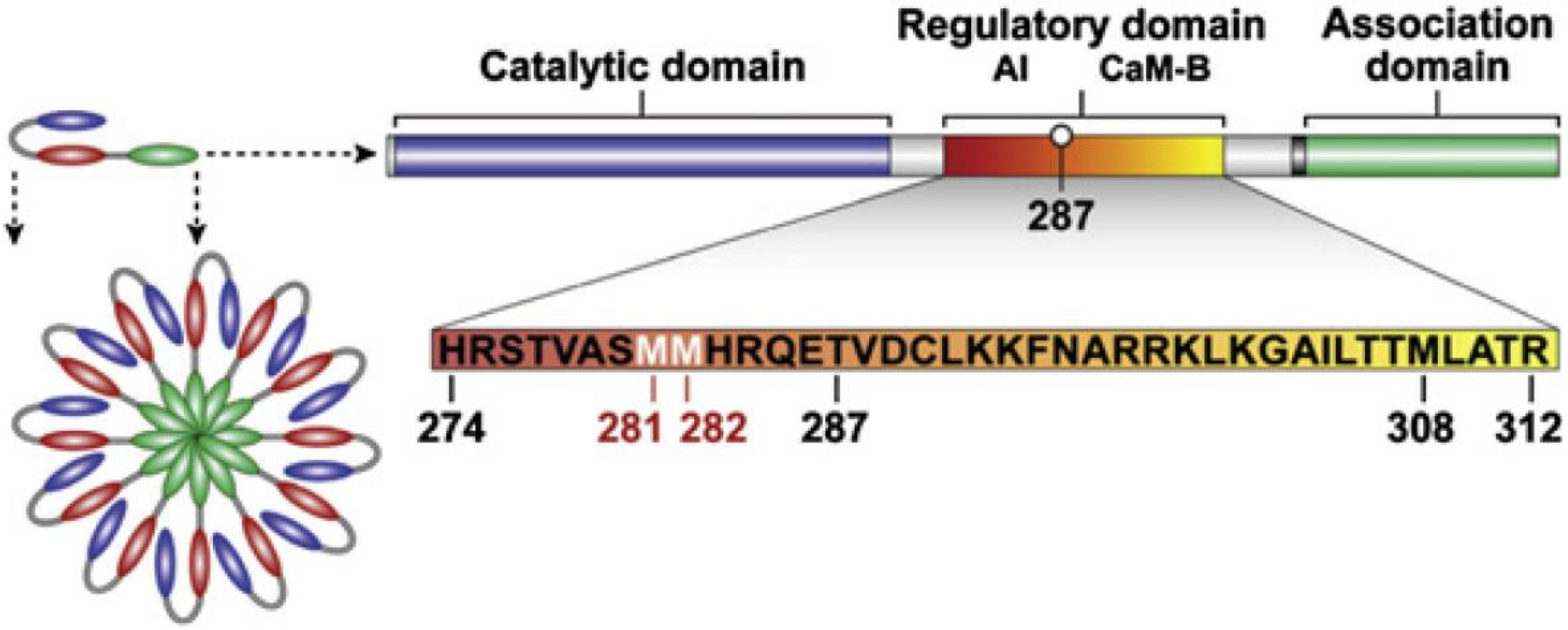


H_2O_2 induced APD prolongation is mediated by CaMKII





CaMKII



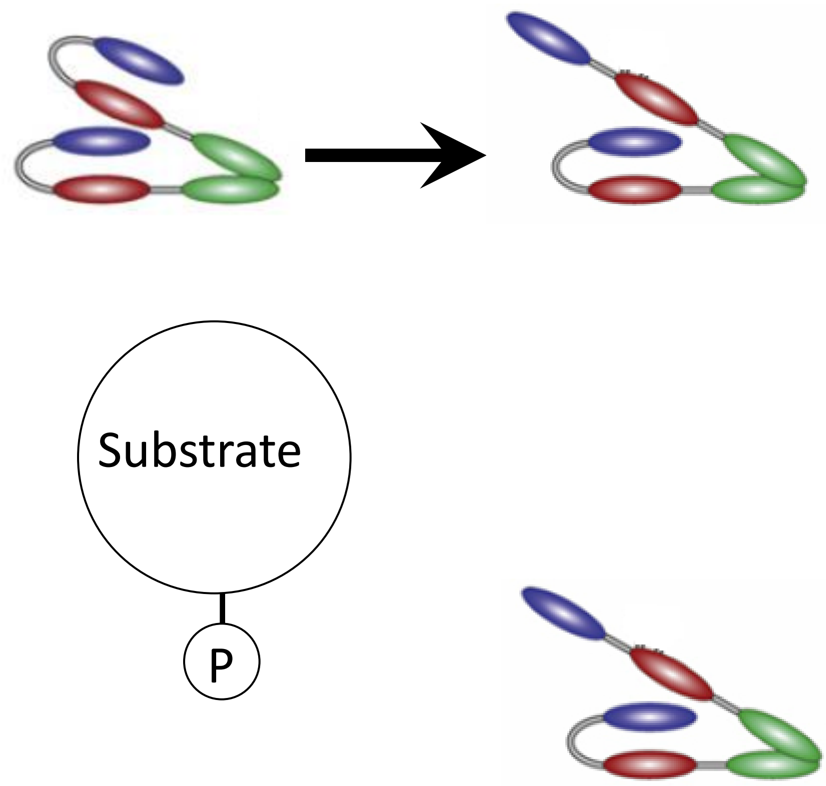
Catalytic domain
Regulatory domain
AI
CaM-B
Association
domain
287
HRSTVASMMHRQETVDCLKKFNARRKLKGAILTTMLATR
274
281
282
287
308
312

Mechanism of CaMKII

Activation

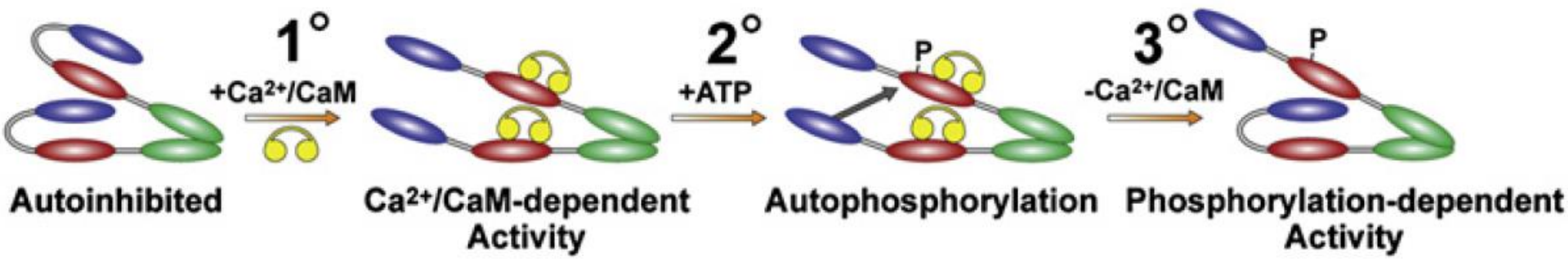
↓

Catalytic activity

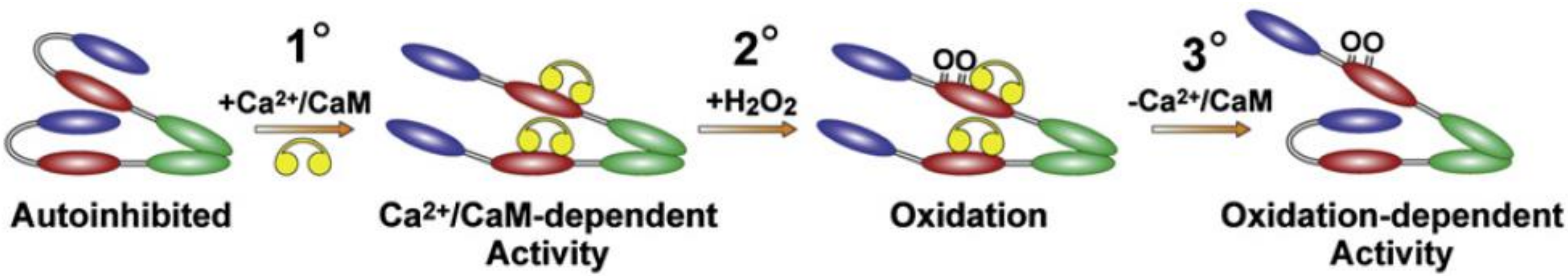


Activation of CaMKII

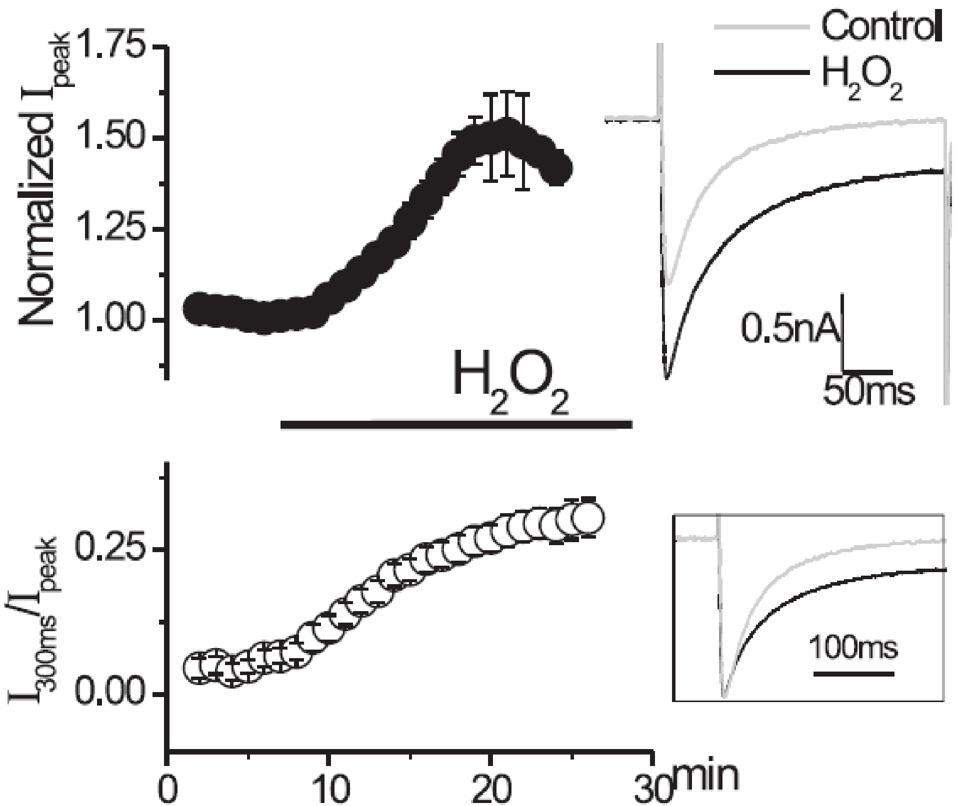
Autophosphorylation

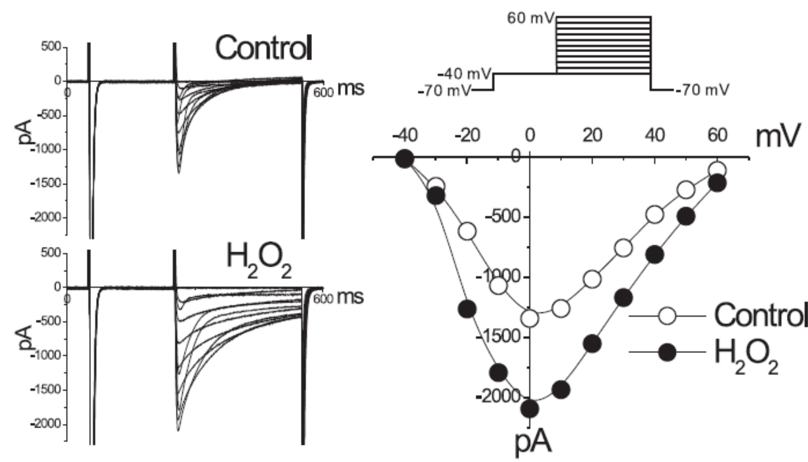


Oxidation

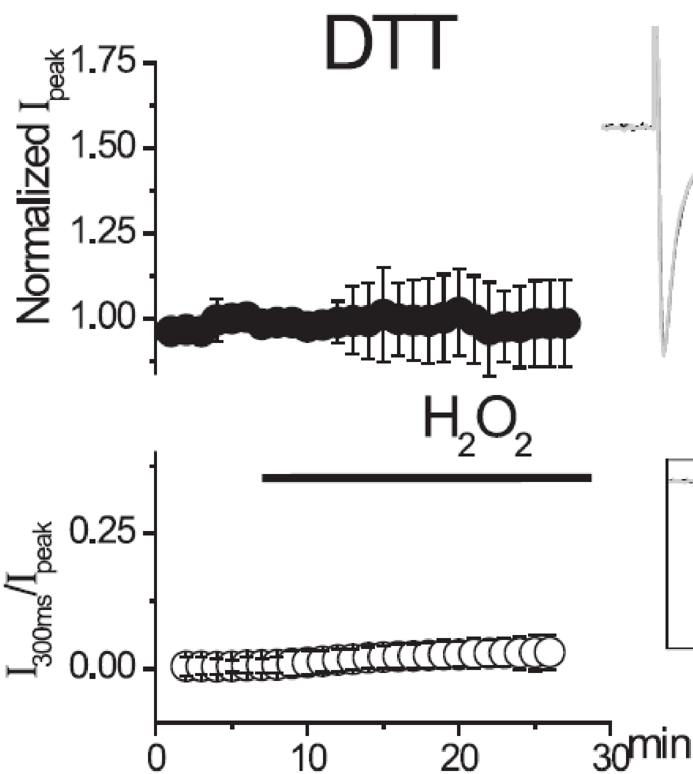


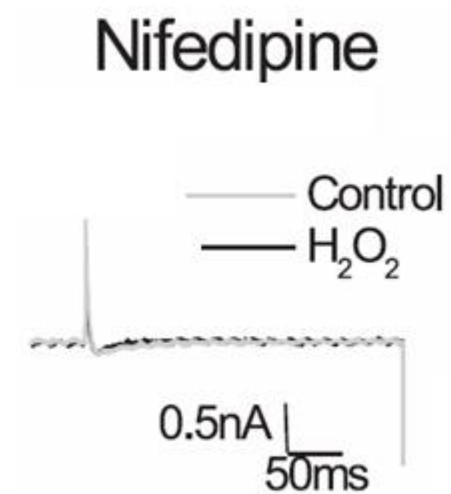
Facilitation of $I_{Ca,L}$ by H_2O_2



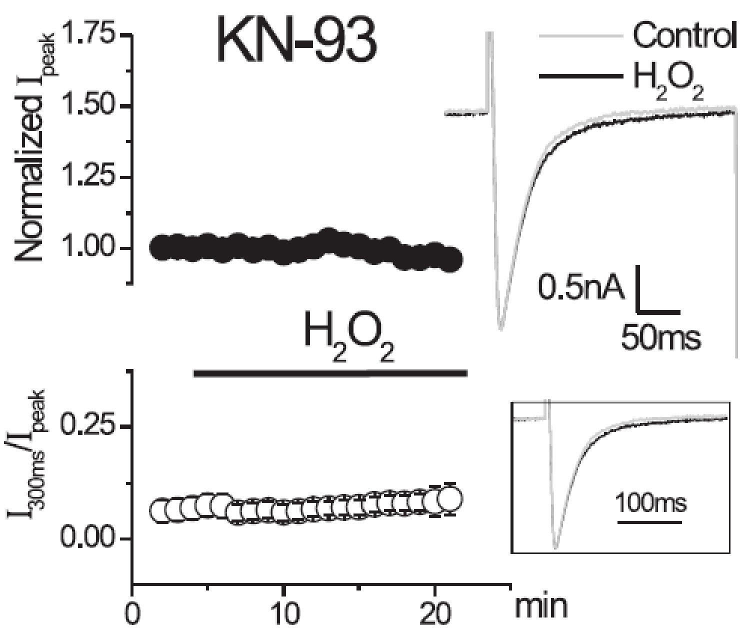


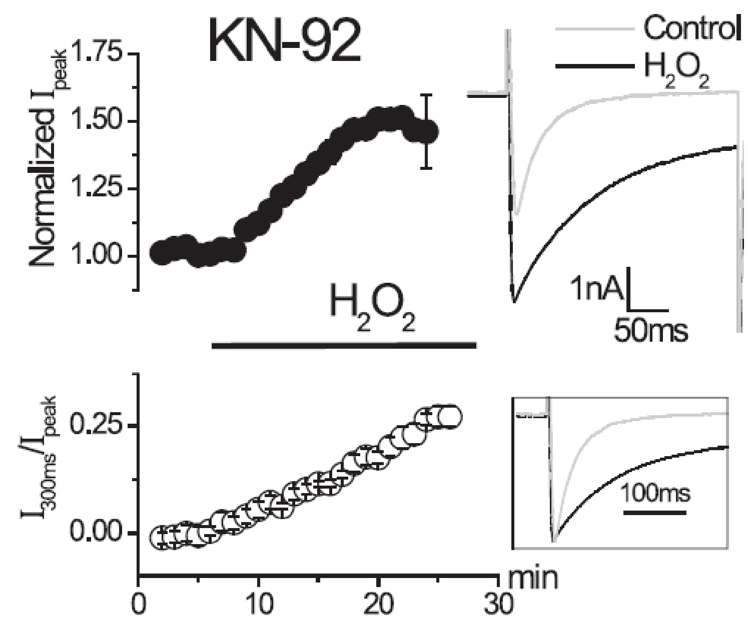
Oxidation-dependent Facilitation of $I_{Ca,L}$ (ODF)



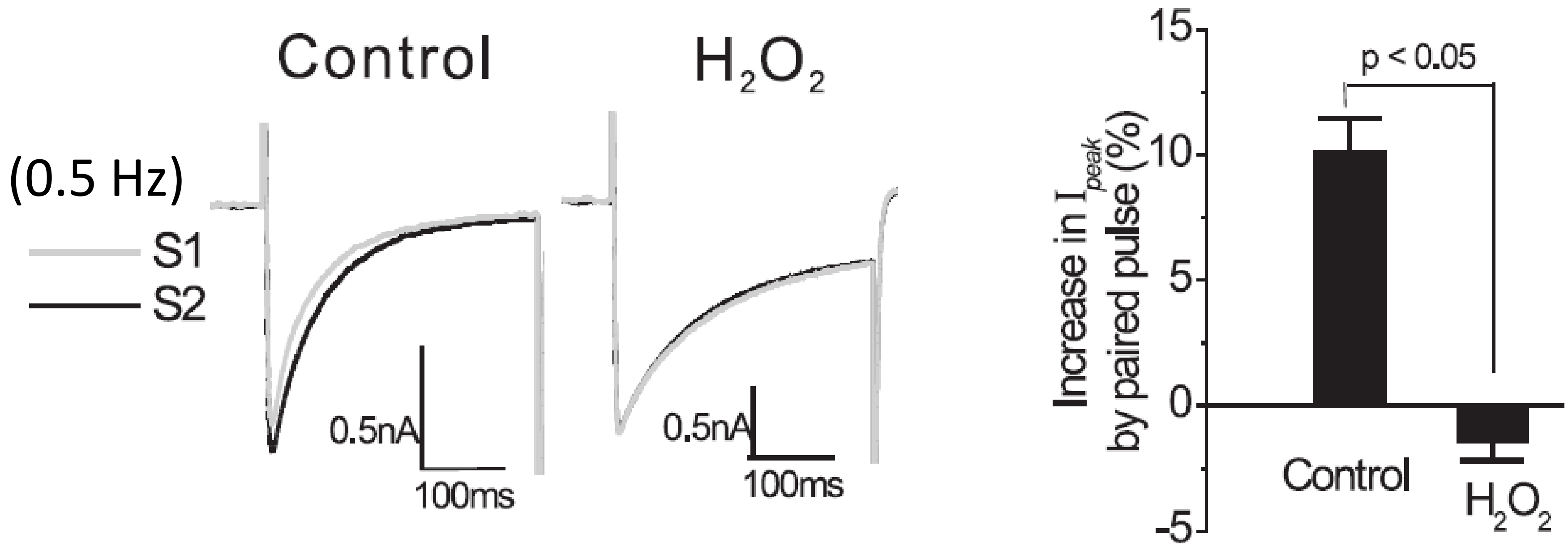


ODF mediated by CaMKII

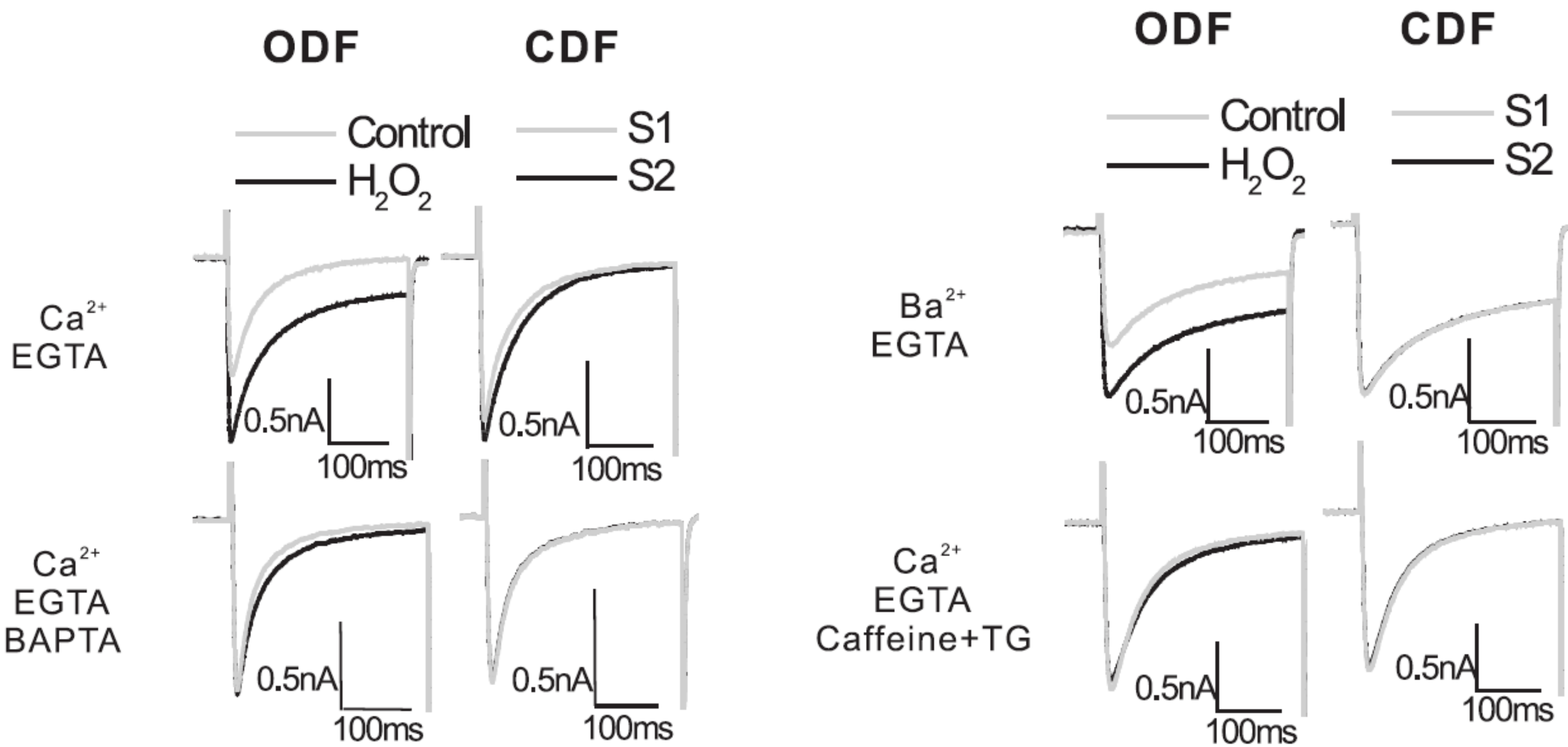




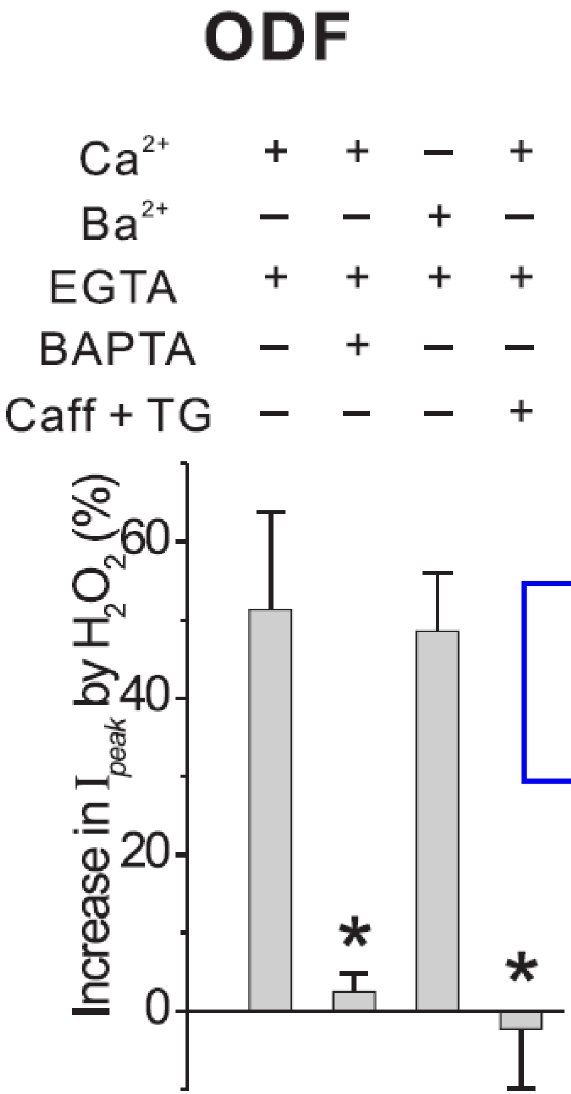
Occlusion of ODF with CDF
(Ca^{2+} dependent facilitation)



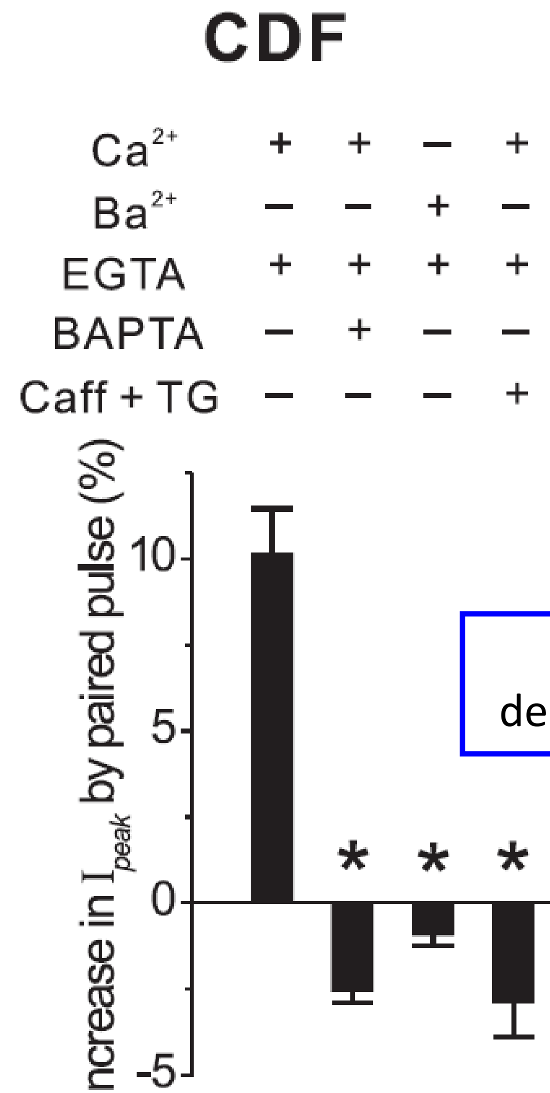
Ca^{2+} Source of ODF & CDF



Ca^{2+} Source of ODF & CDF

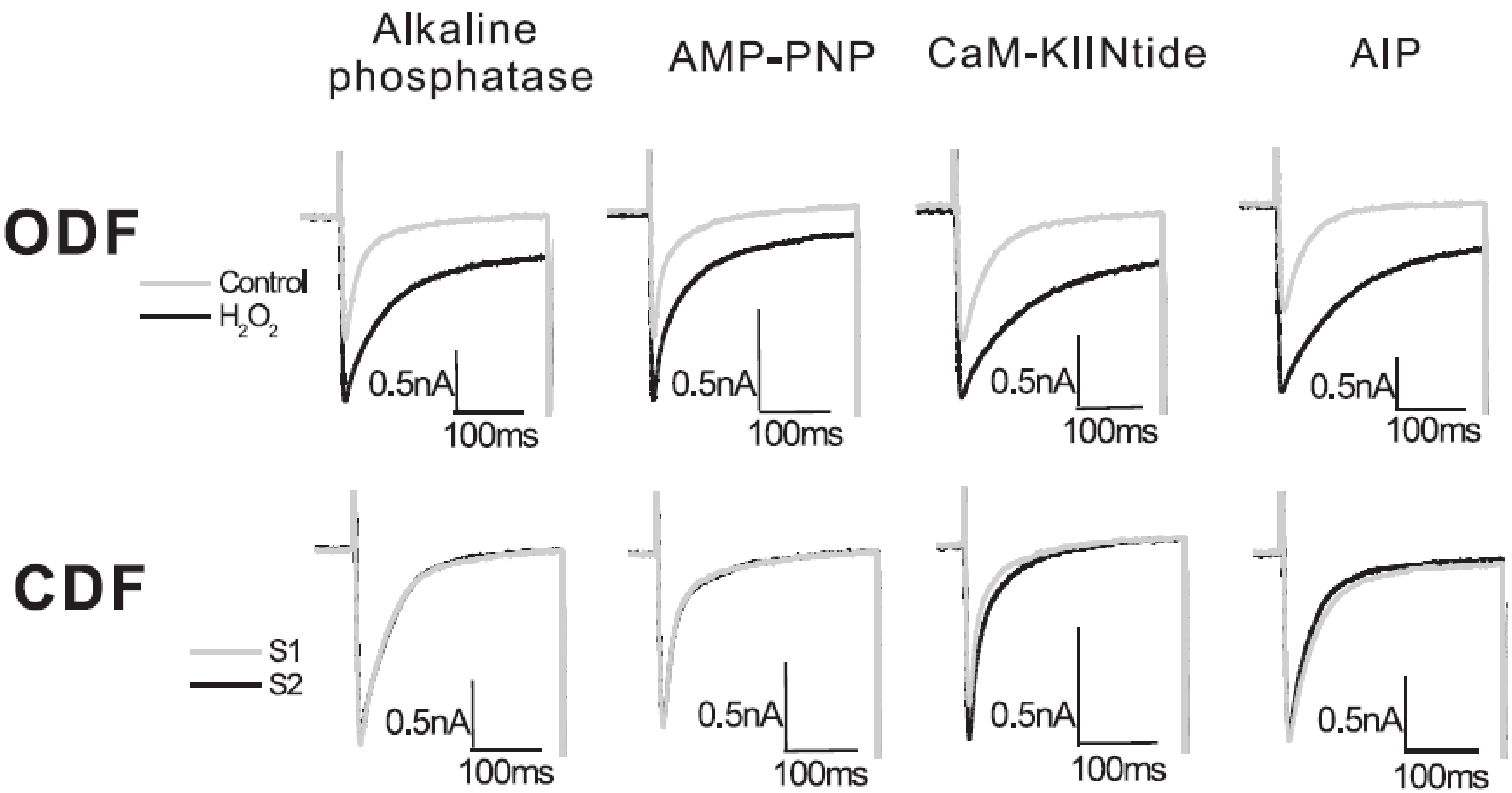


Spontaneous
SR Ca^{2+} release
dependent

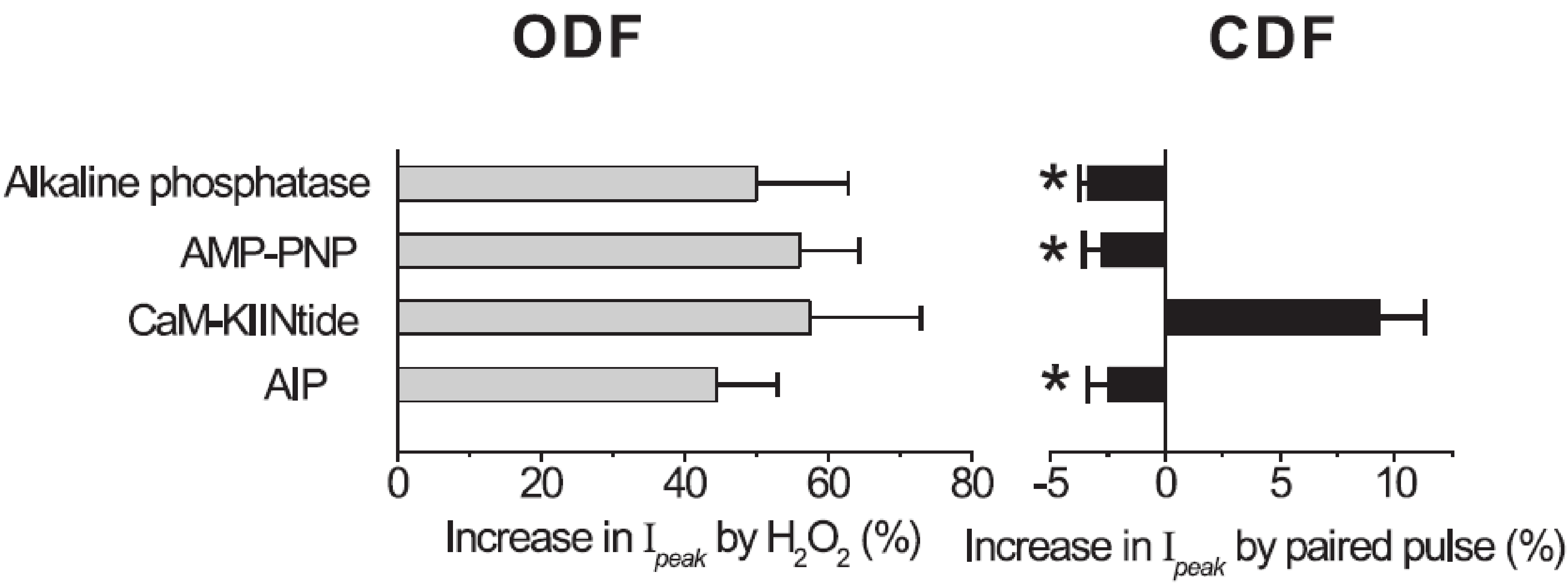


CICR
dependent

Phosphorylation in ODF & CDF (Autophosphorylation, Catalytic activity)



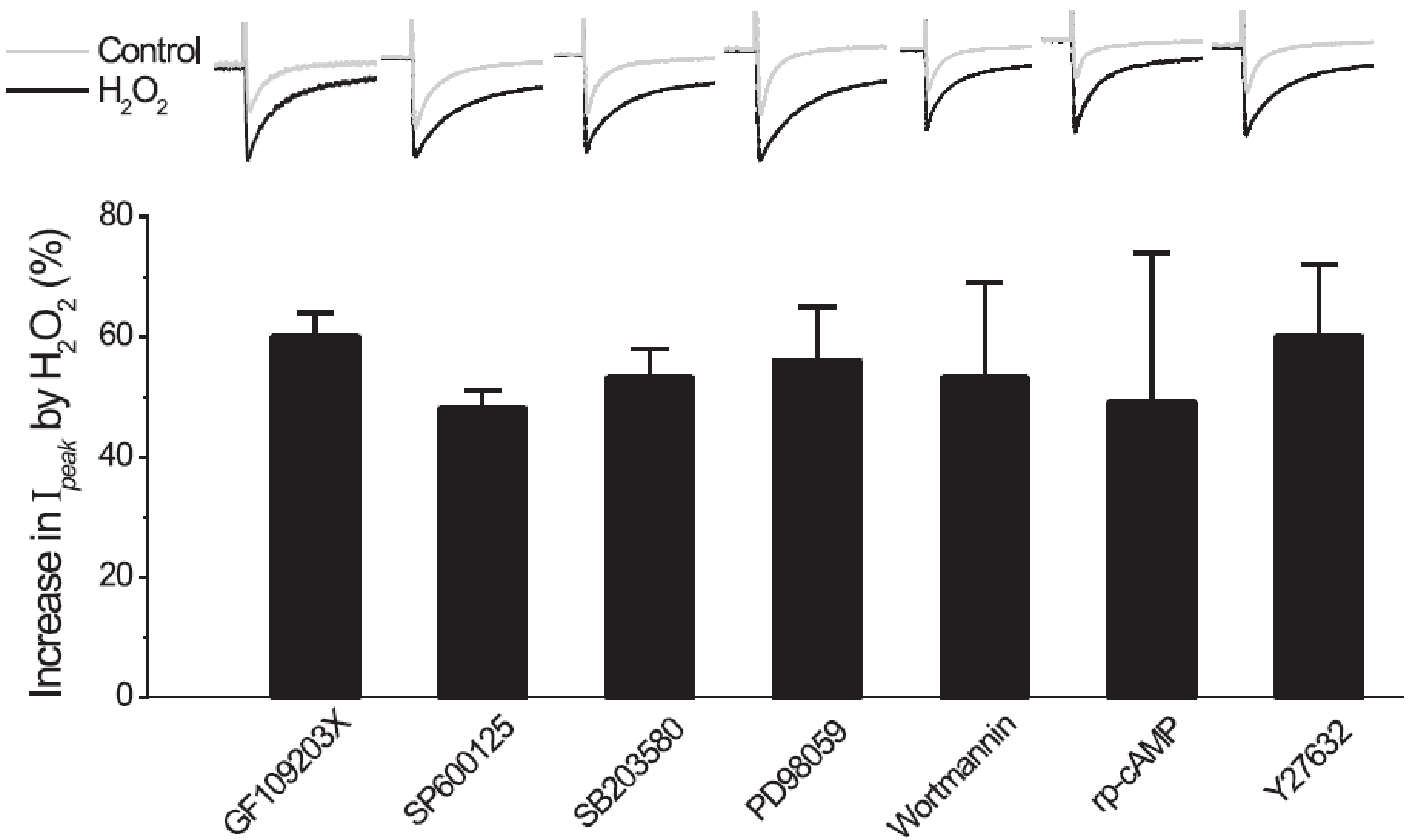
Phosphorylation in ODF & CDF



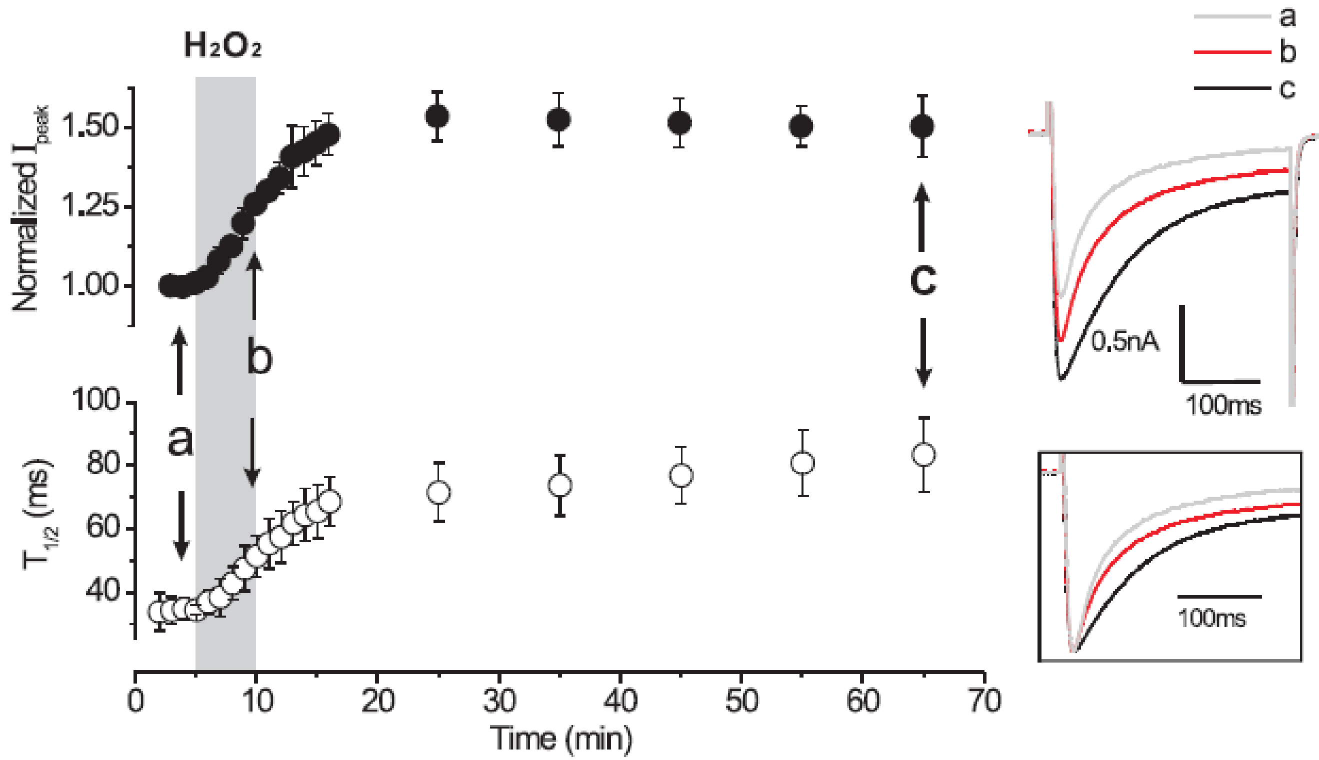
ODF; Autophosphorylation-independent non-catalytic reaction

CDF; Autophosphorylation-dependent non-catalytic reaction

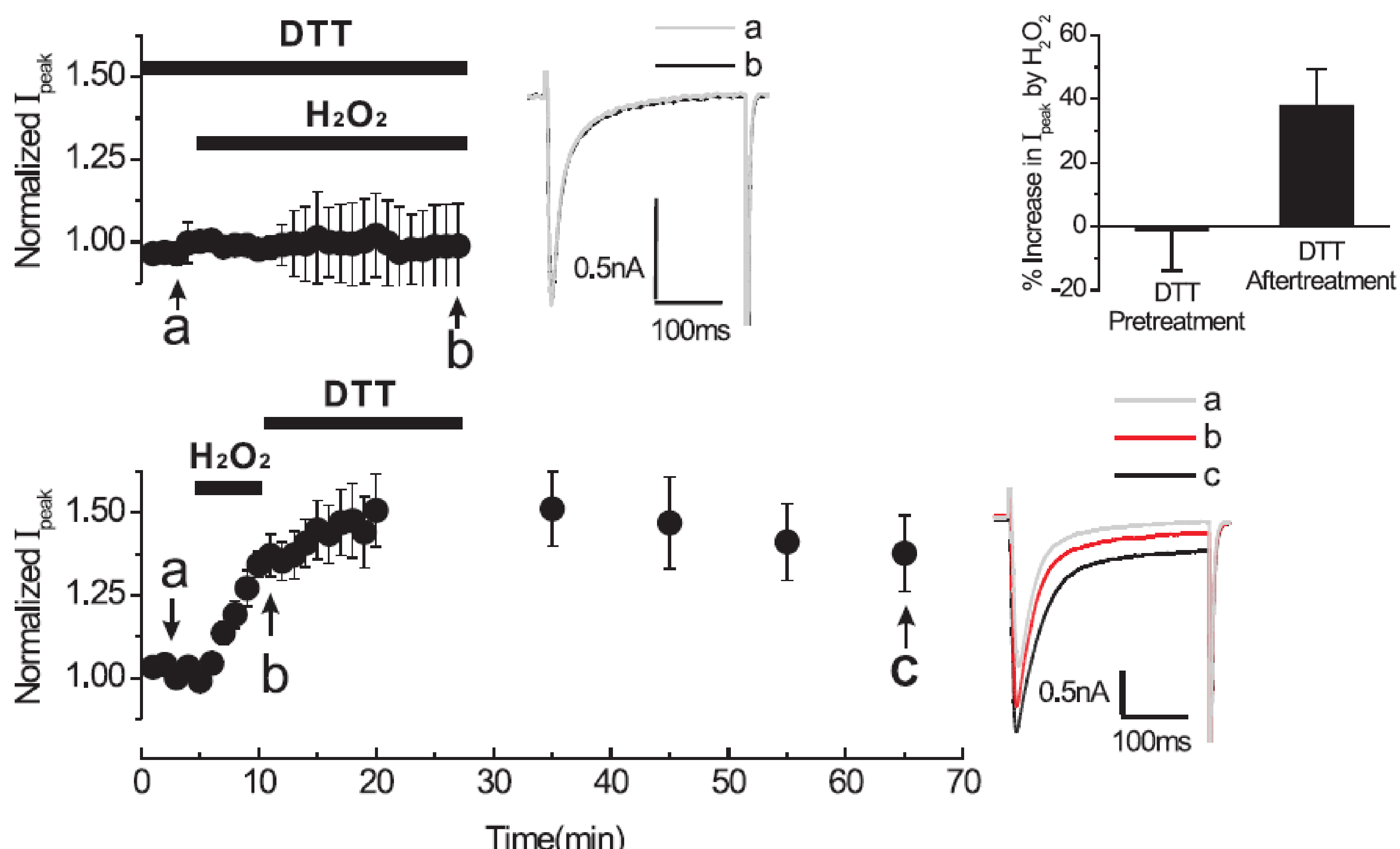
The effects of kinase inhibitors related to ROS on ODF



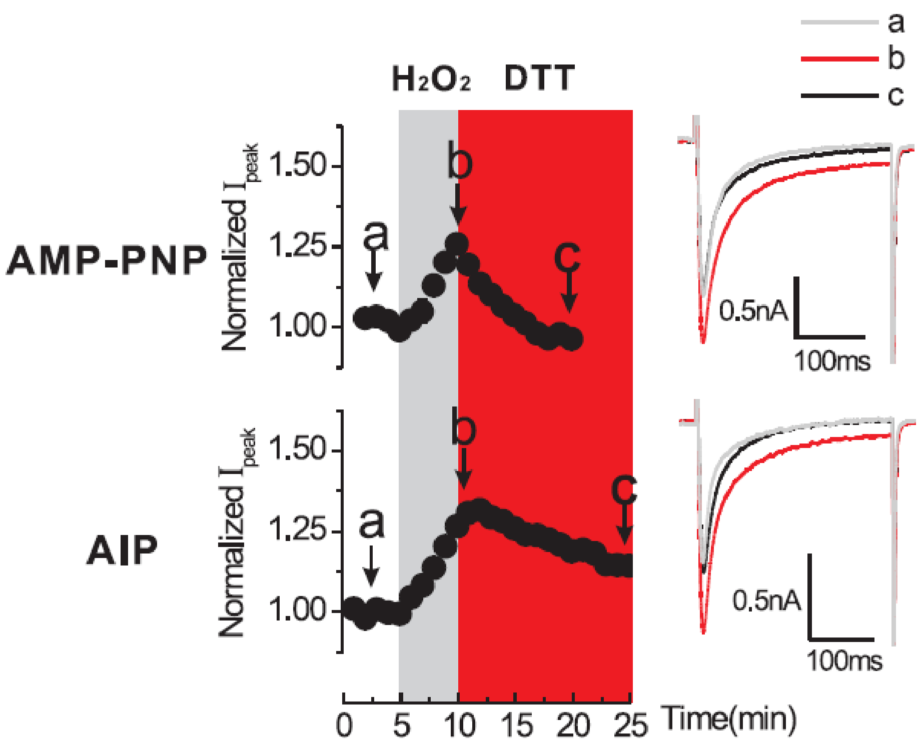
Long term potentiation (LTP)



The effct of DTT on LTP



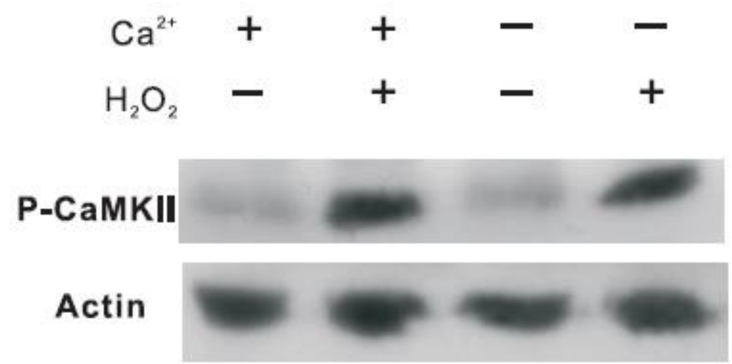
Oxidation-dependent Autophosphorylation of CaMKII



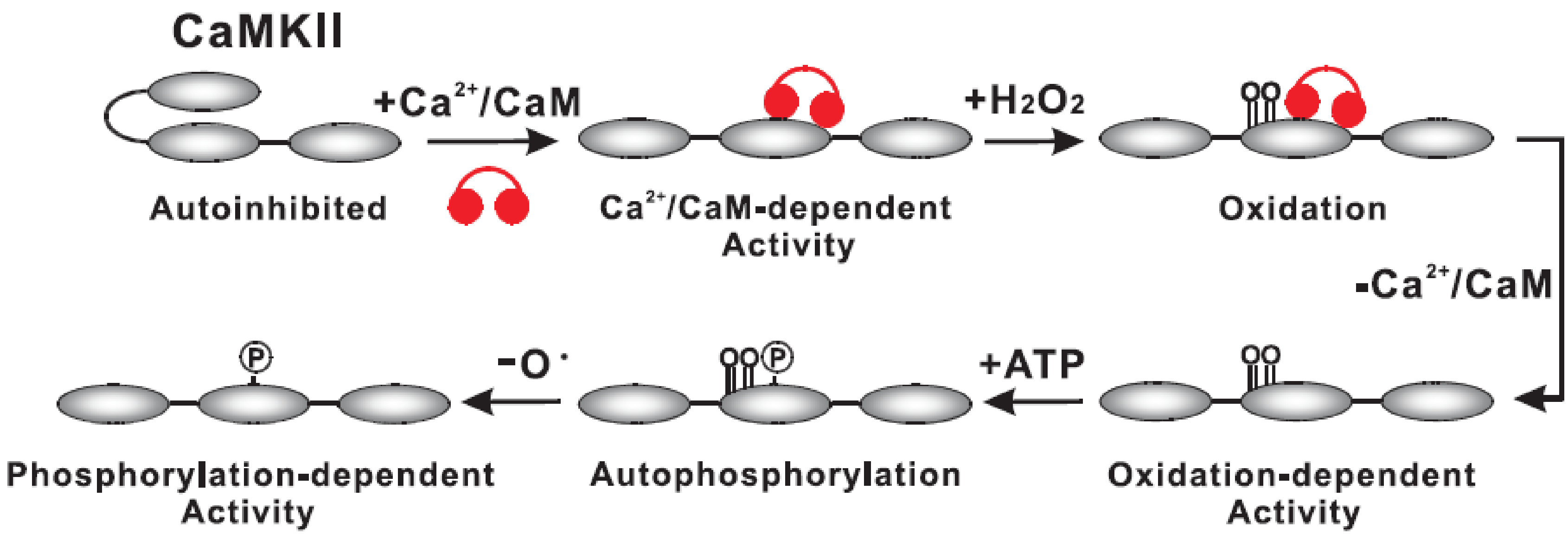
Langendorff perfusion with Solutions (+/- Ca^{2+}, +/- H_2O_2)

→Cut & grind vent. tissue

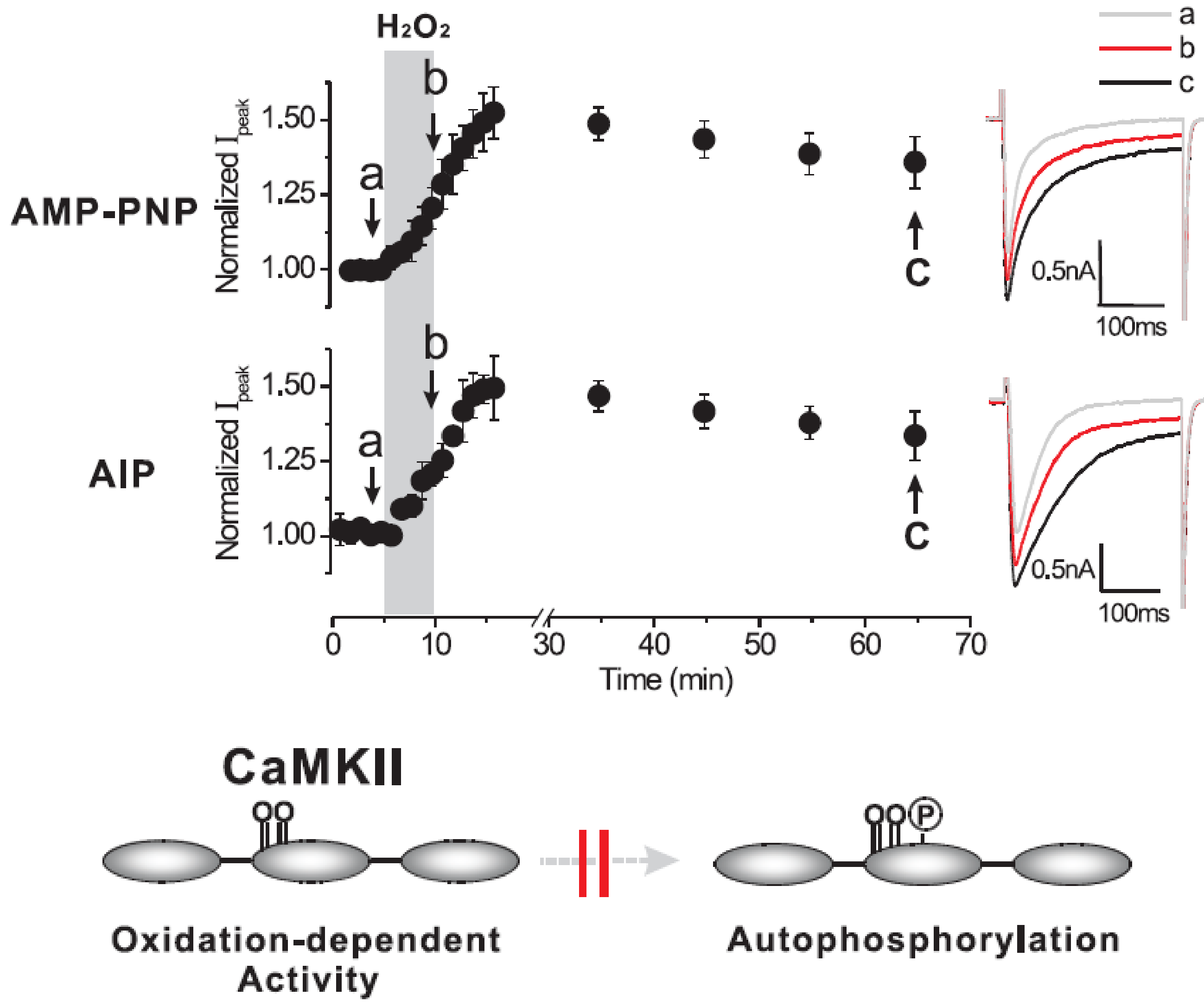
→Western blot



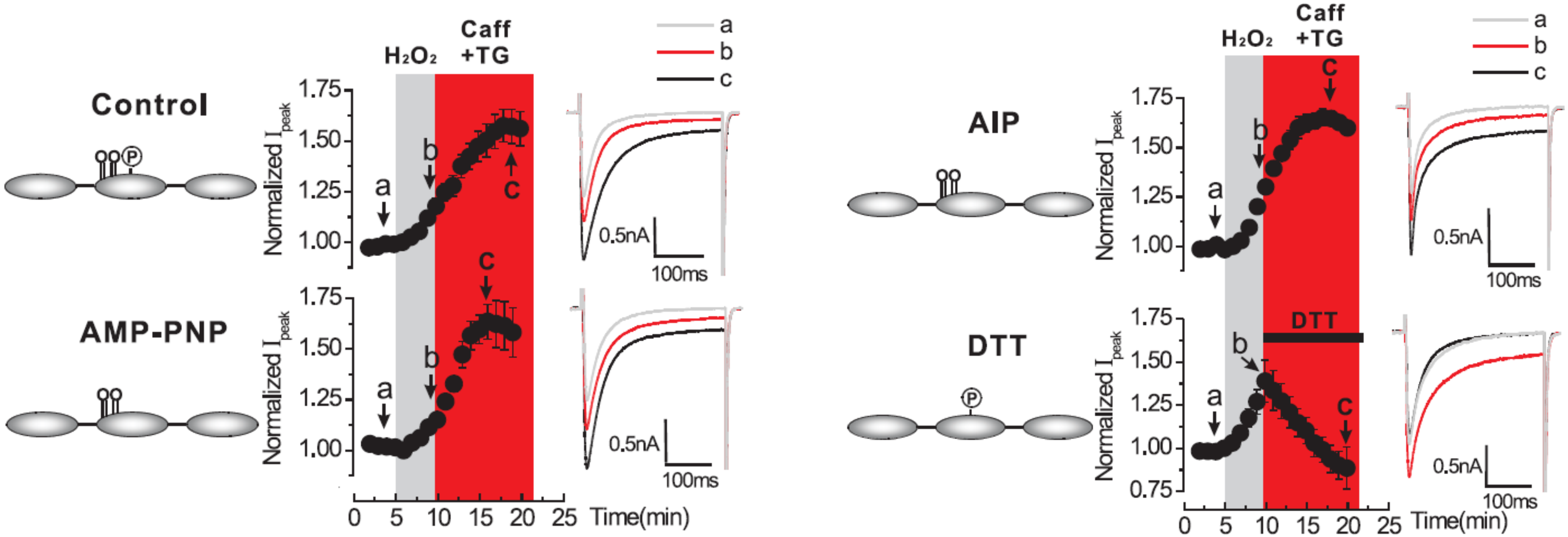
The New Mechanism of CaMKII Activation

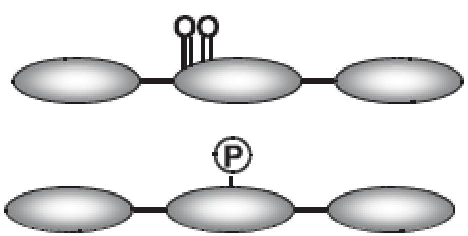


CaMKII Oxidation alone in LTP



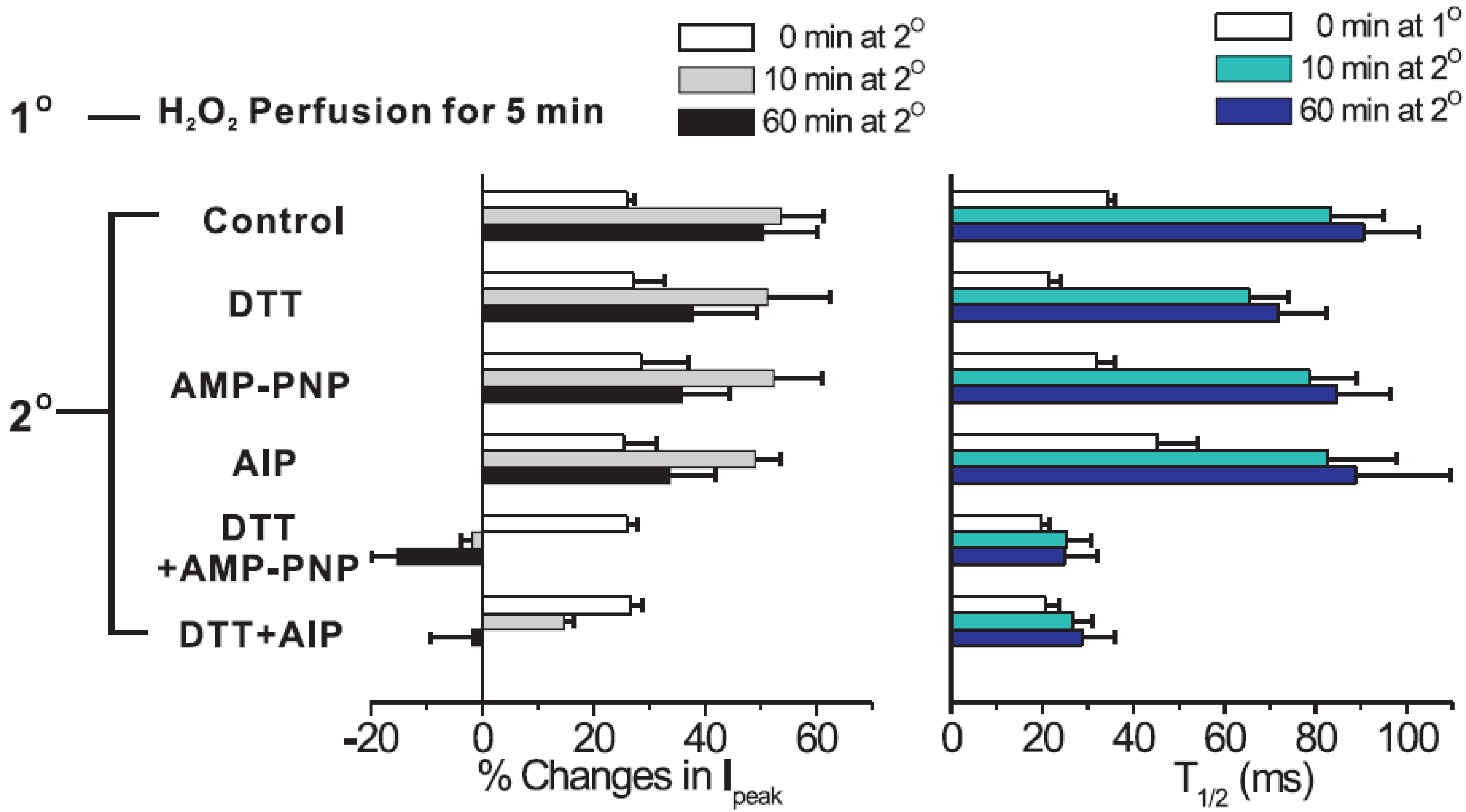
SR Ca^{2+} Dependency



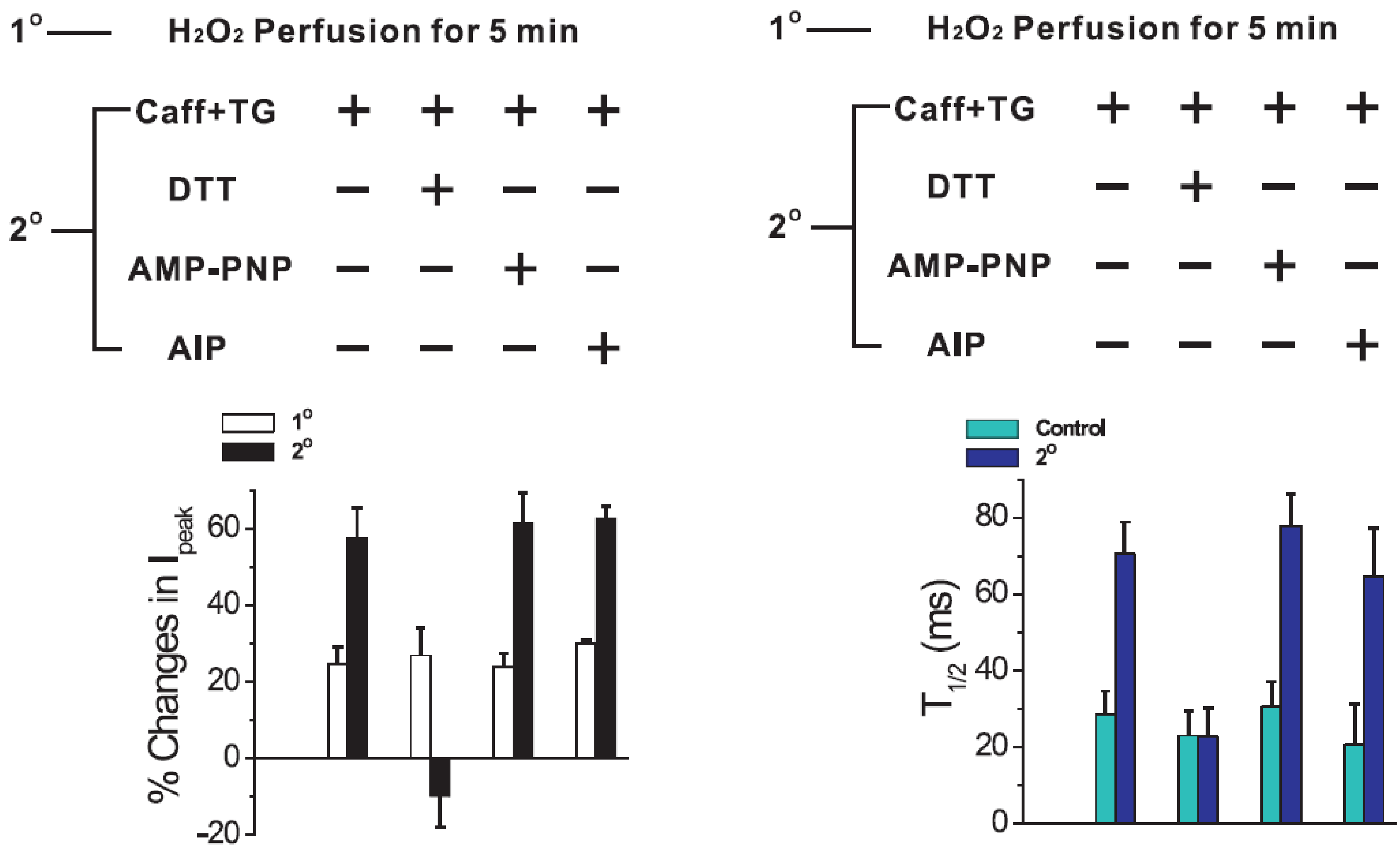


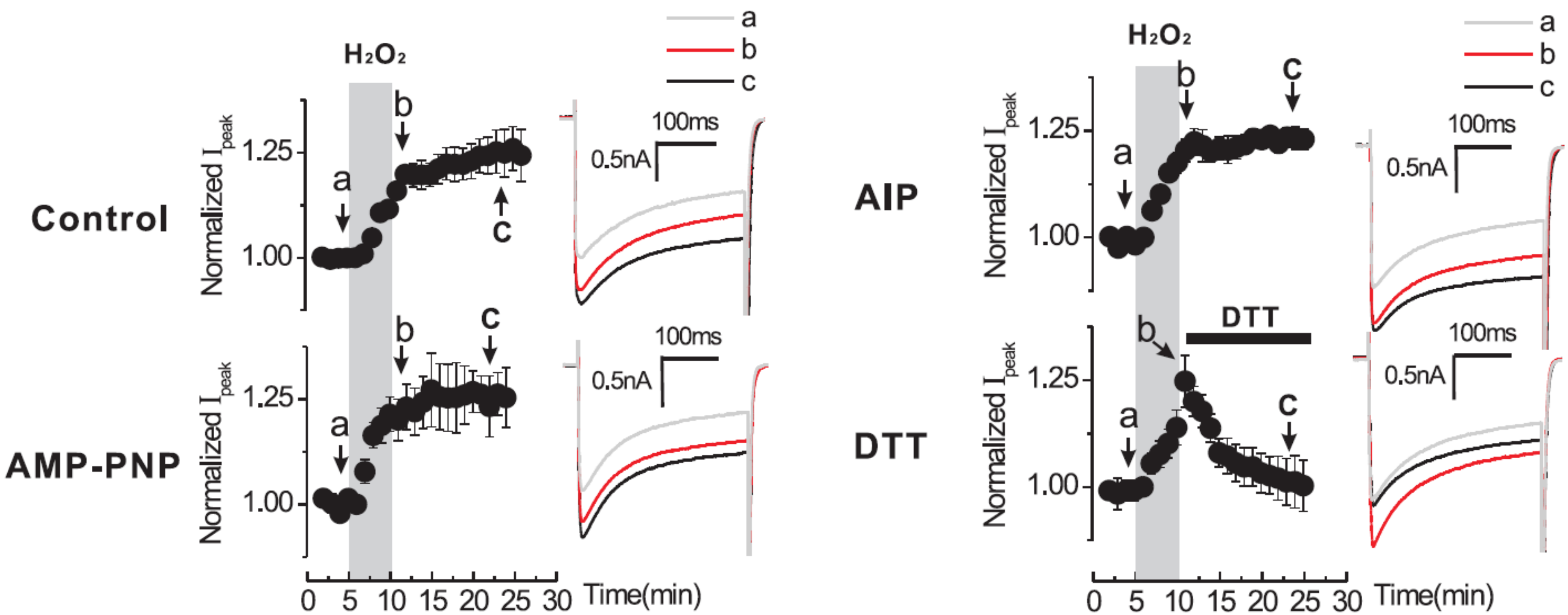
--- SR Ca^{2+} release independent

--- SR Ca^{2+} release dependent

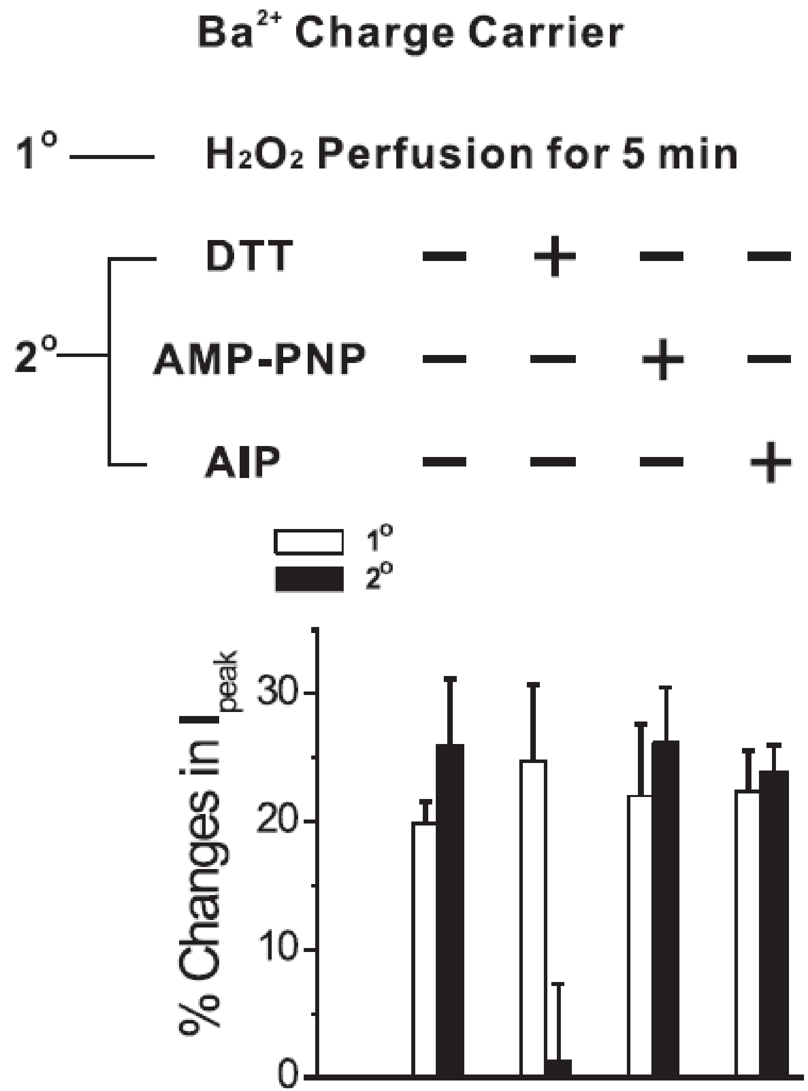


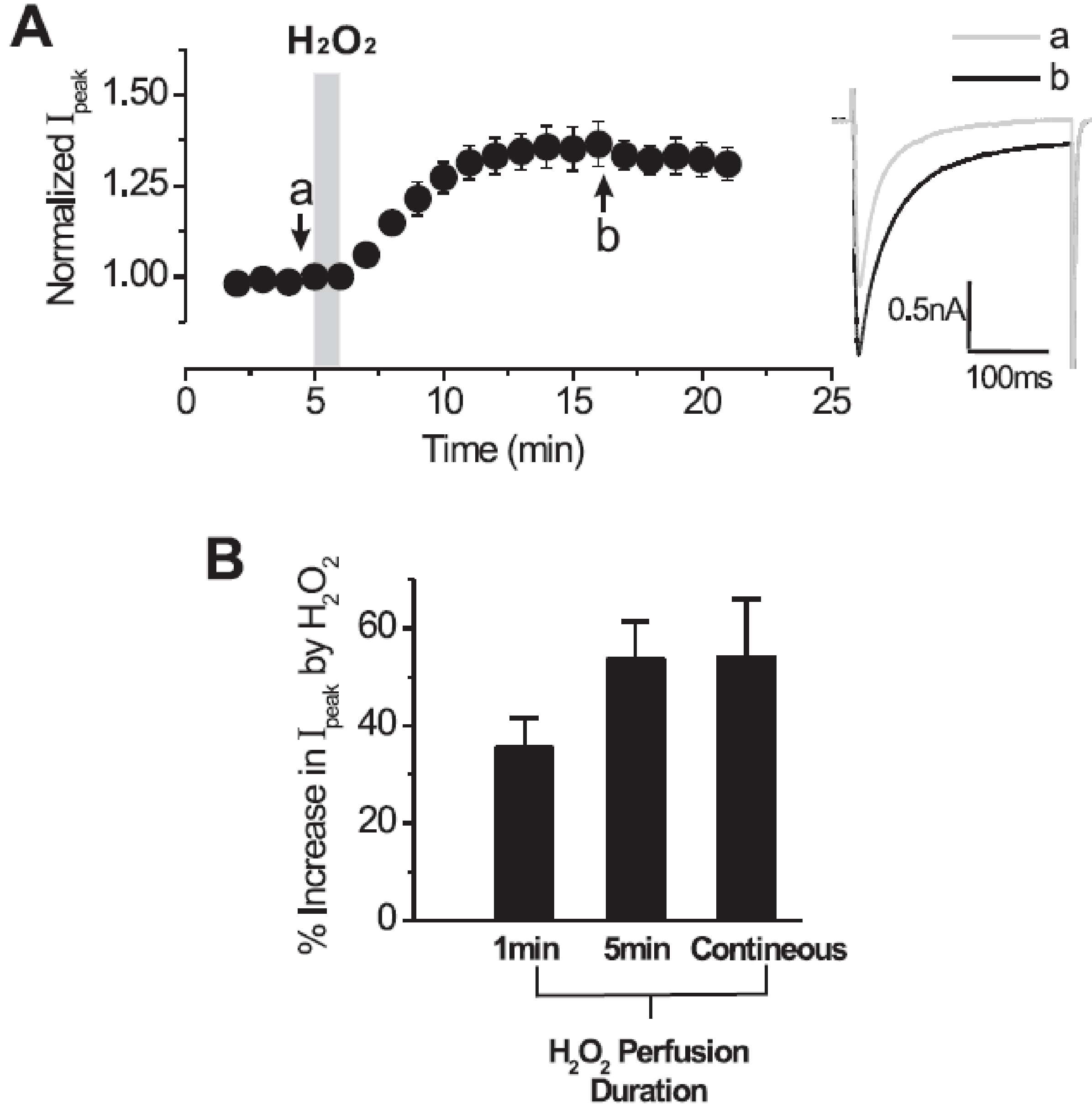
0 min at 2°
10 min at 2°
60 min at 2°
0 min at 1°
10 min at 2°
60 min at 2°
1° — H2O2 Perfusion for 5 min
2°
Control
DTT
AMP-PNP
AIP
DTT +AMP-PNP
DTT+AIP
-20 0 20 40 60
% Changes in Ipeak
0 20 40 60 80 100
T1/2 (ms)


1° H2O2 Perfusion for 5 min
2°
Caff+TG + + + +
DTT − + − −
AMP-PNP − − + −
AIP − − − +
1°
2°
% Changes in Ipeak
60
40
20
0
-20
1° H2O2 Perfusion for 5 min
2°
Caff+TG + + + +
DTT − + − −
AMP-PNP − − + −
AIP − − − +
Control
2°
T1/2 (ms)
80
60
40
20
0

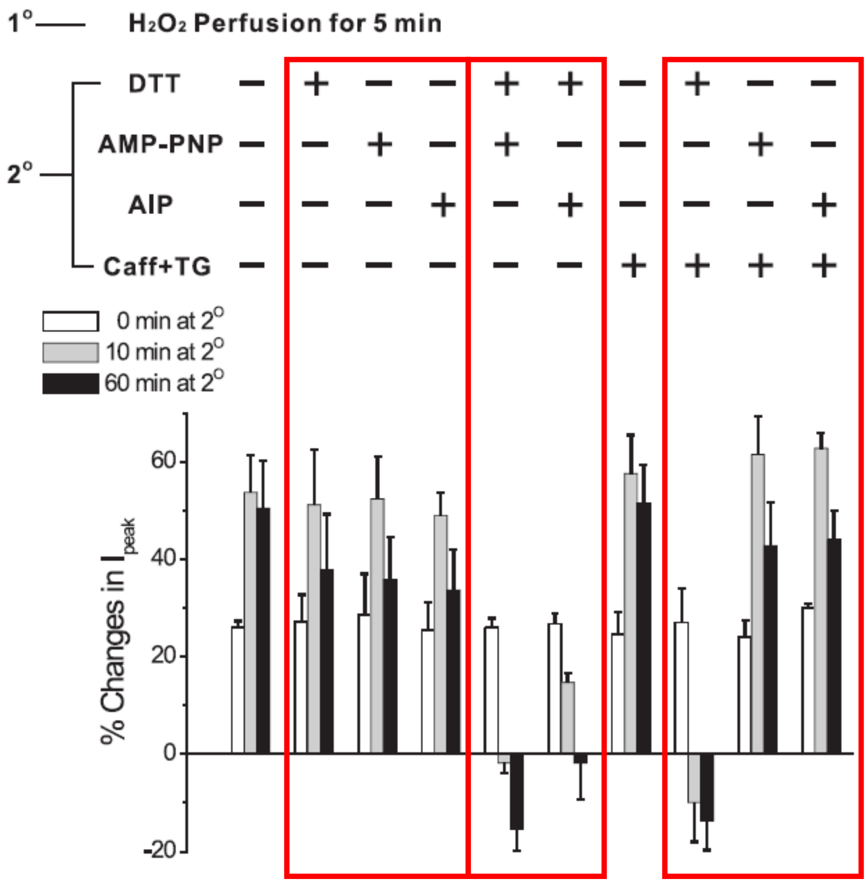


Control
AMP-PNP
AIP
DTT
H2O2
DTT
Normalized Ipeak
1.25
1.00
a
b
c
100ms
0.5nA
0 5 10 15 20 25 30
Time(min)

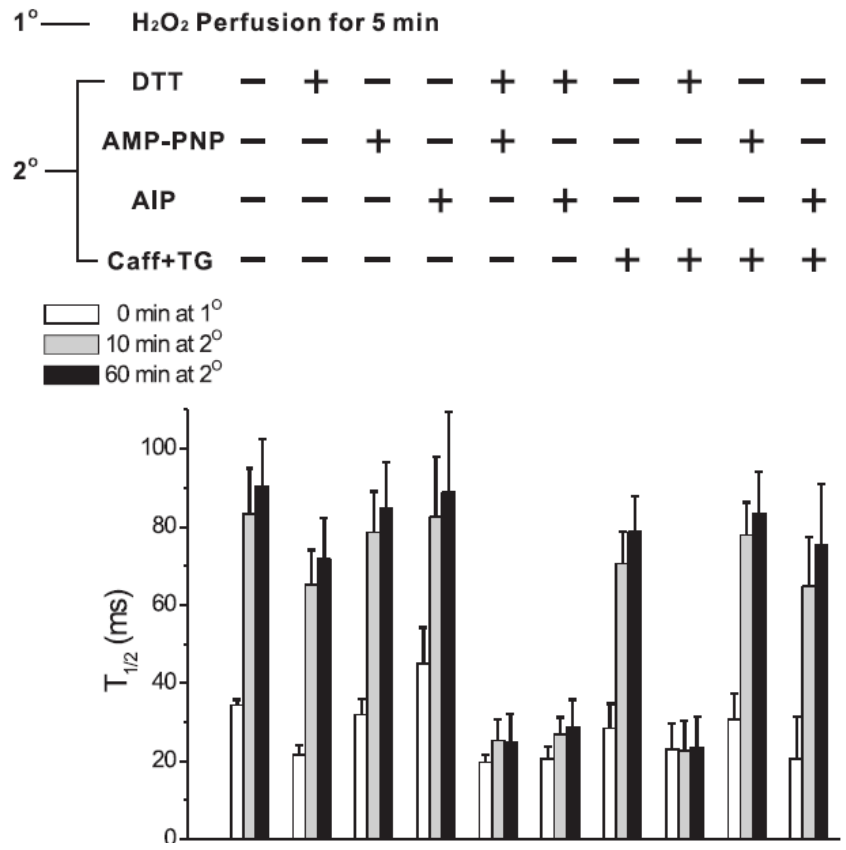

Ba2+ Charge Carrier
1° H2O2 Perfusion for 5 min
2°
DTT − + − −
AMP-PNP − − + −
AIP − − − +
1°
2°
% Changes in Ipeak
0
10
20
30


A
H₂O₂
Normalized I peak
1.50
1.25
1.00
a
b
0
5
10
15
20
25
Time (min)
a
b
0.5nA
100ms
B
% Increase in I peak by H2O2
60
40
20
0
1min
5min
Contineous
H2O2 Perfusion Duration

Summary

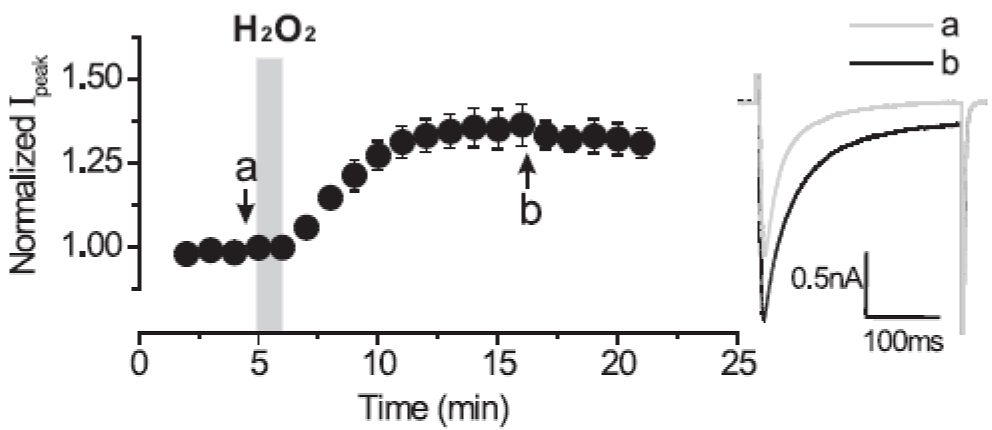


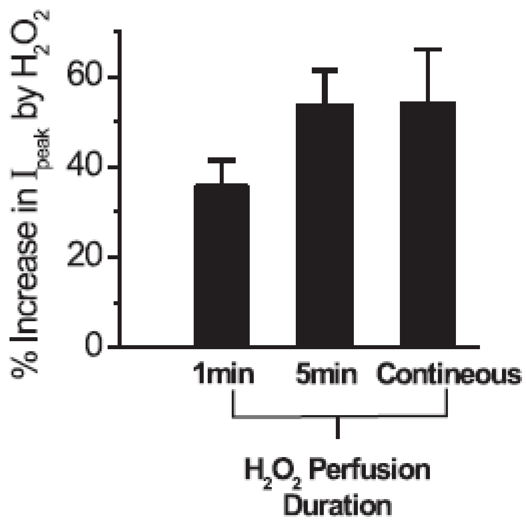
1° H2O2 Perfusion for 5 min
2°
DTT
AMP-PNP
AIP
Caff+TG
0 min at 2°
10 min at 2°
60 min at 2°
% Changes in I peak



1° H2O2 Perfusion for 5 min
2°
DTT
AMP-PNP
AIP
Caff+TG
0 min at 1°
10 min at 2°
60 min at 2°
T 1/2 (ms)

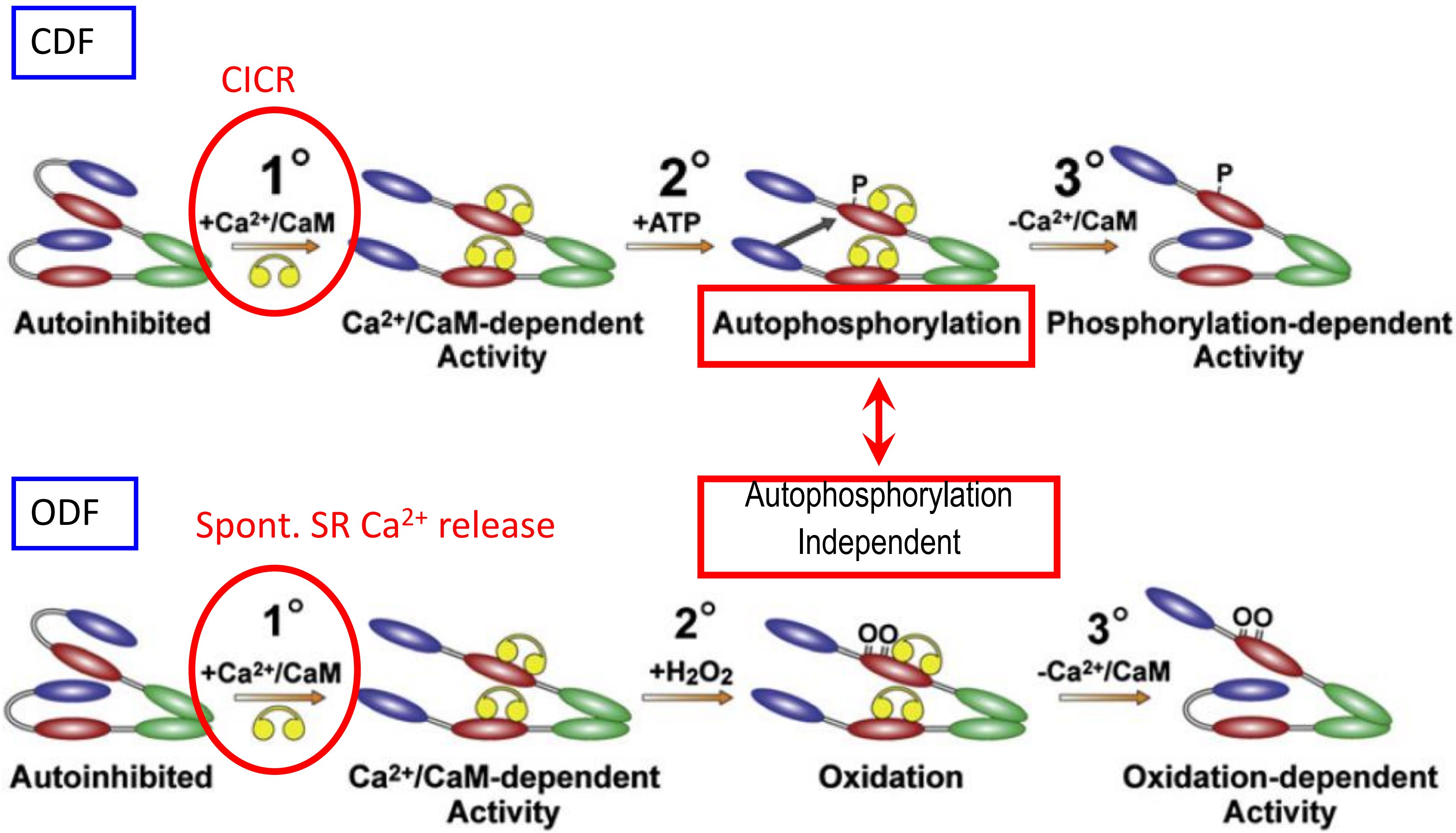
The Effect of Duration of H_2O_2 Perfusion on LTP





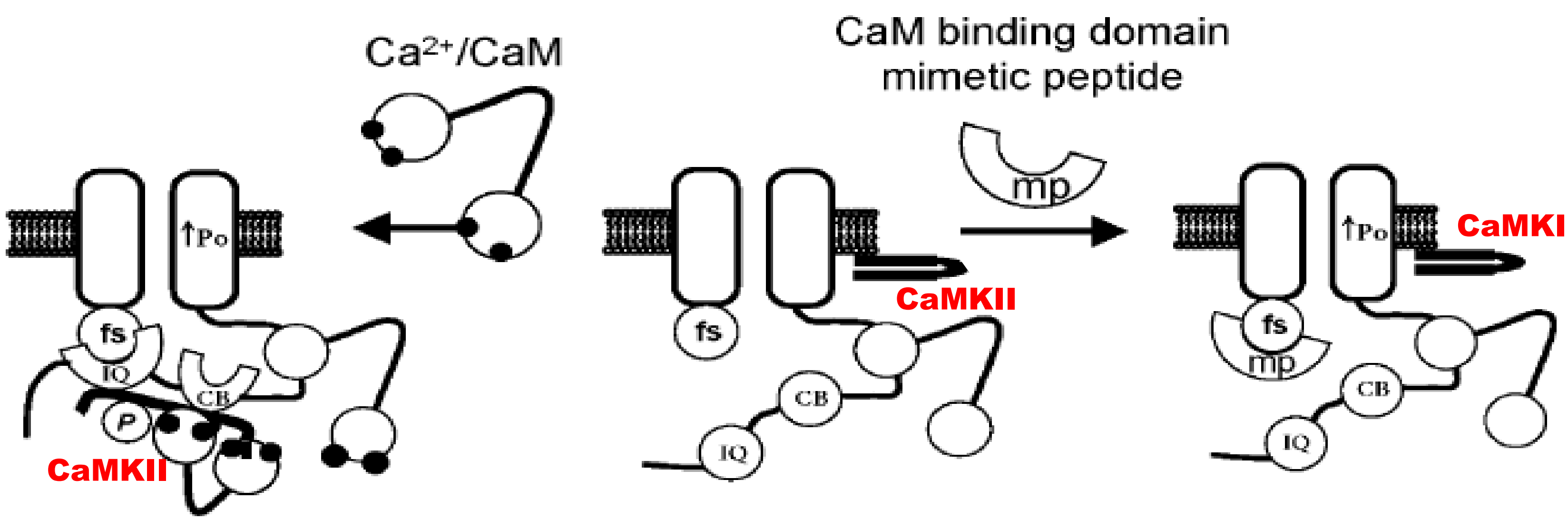
Discussion

Activation of CaMKII

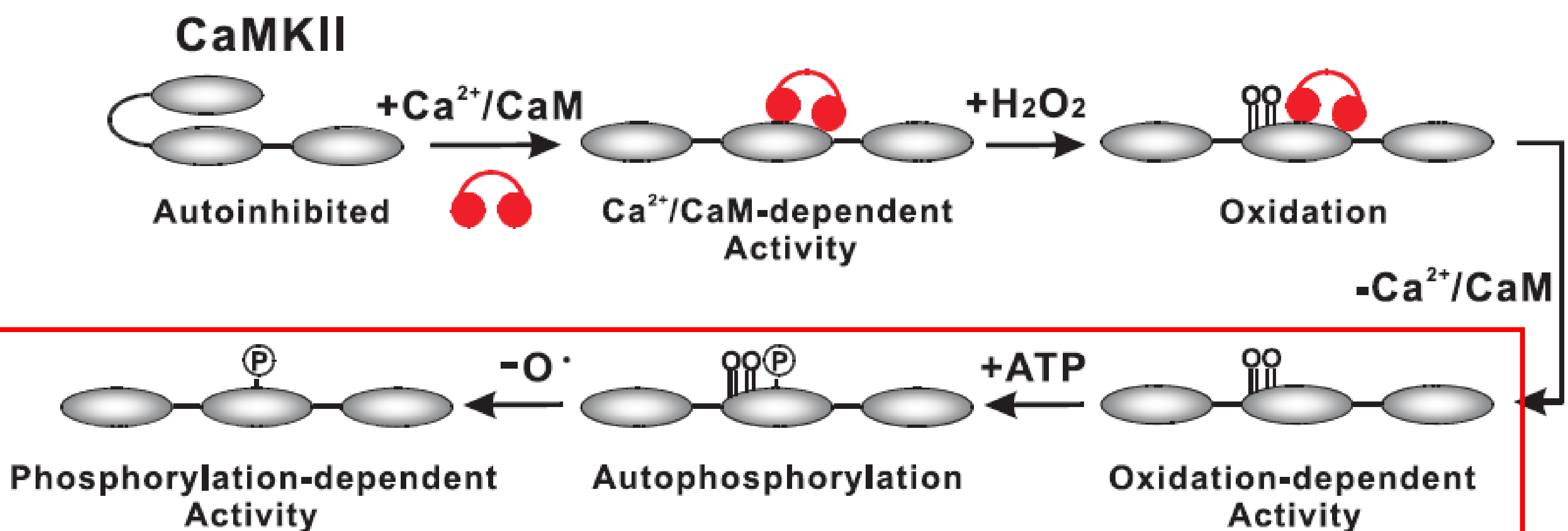


Interaction of CaMKII & L-type Ca^{2+} Channel

Non-catalytic reaction



The New Mechanism of CaMKII Activation



SR Ca^{2+} dependent

SR Ca^{2+} independent

SR Ca^{2+} independent

Long term potentiation: LTP