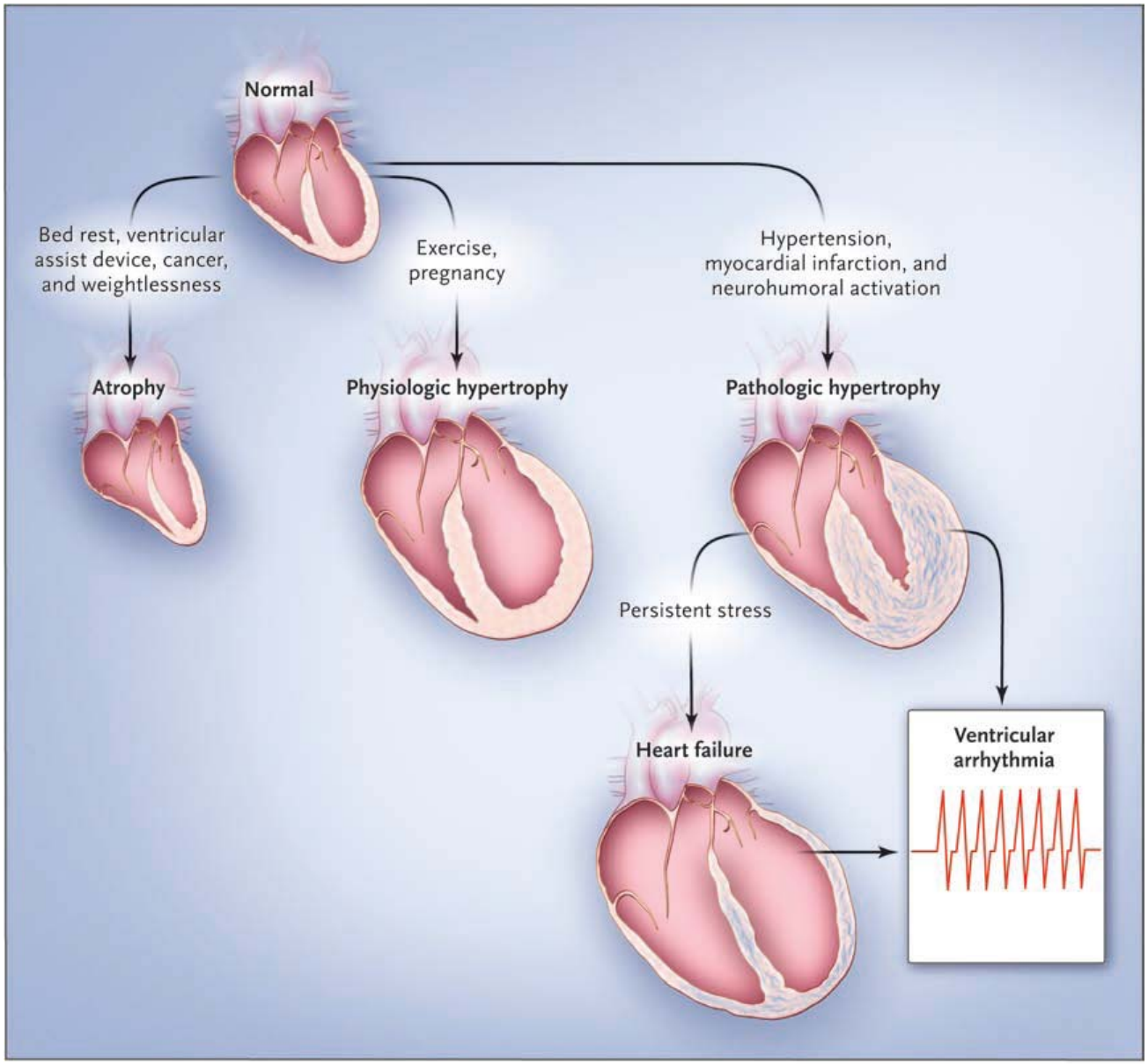
Posttranslational Modification of Histone Deacetylase 2 in Cardiac Hypertrophy

Hyun Kook, MD, PhD

Department of Pharmacology and
Medical Research Center for Gene Regulation
Chonnam National University Medical School,
Gwangju, South Korea

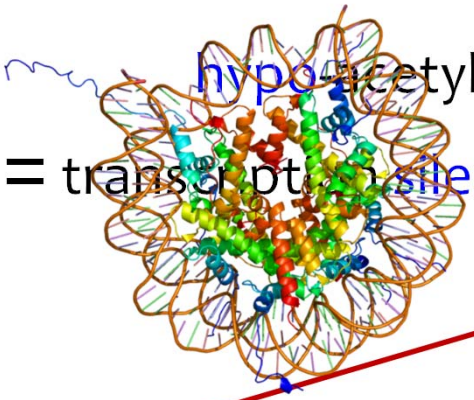
Cardiac Hypertrophy



Hill JA and Olson EN., *N Engl J Med*. **358**:1370–1380 (2008).

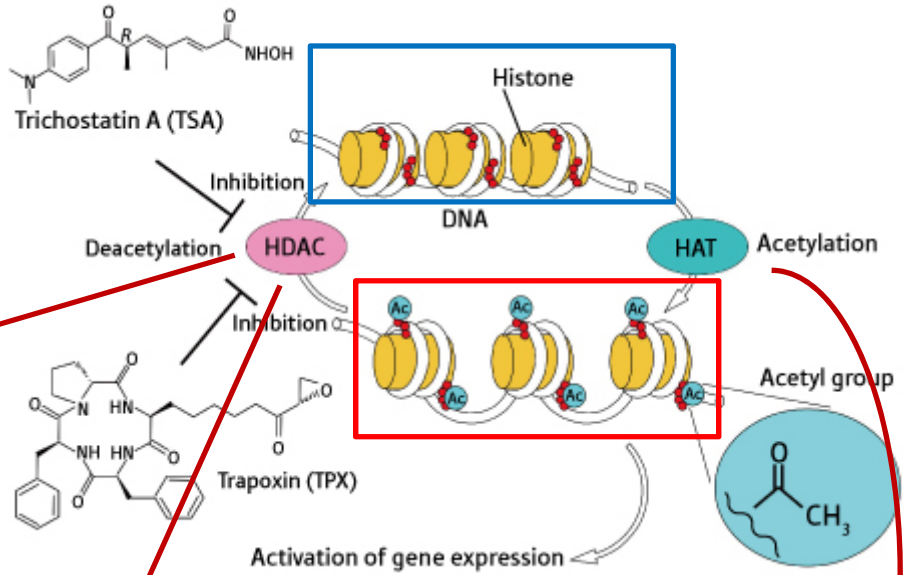
hyper-acetylation = transcription activation

Histone acetylation

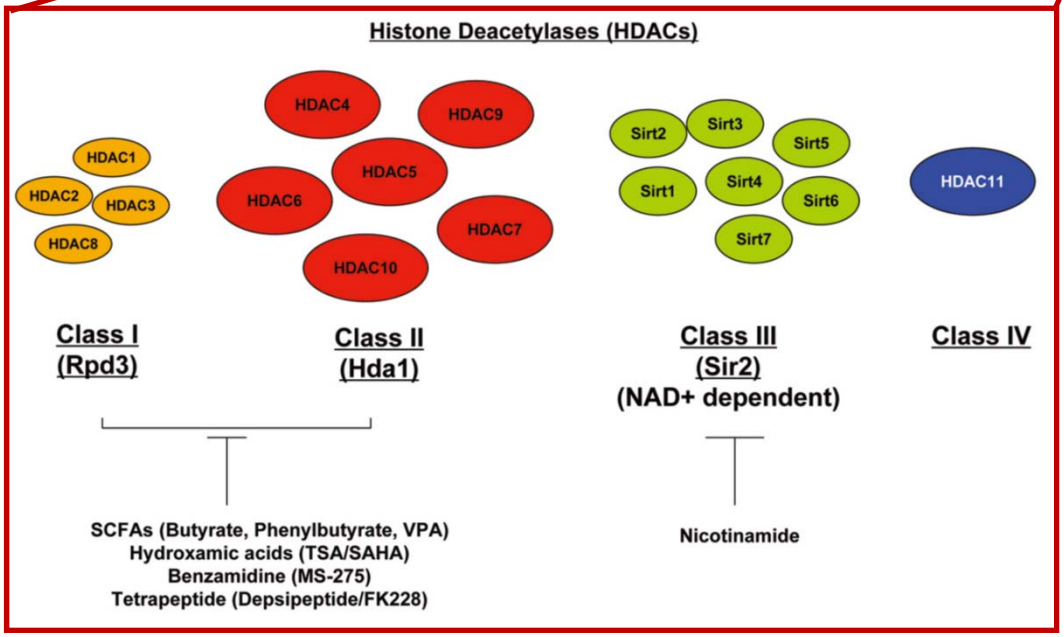


hypo-acetylation

= transcription silencing



http://www.rikenresearch.riken.jp/eng/frontline/5568



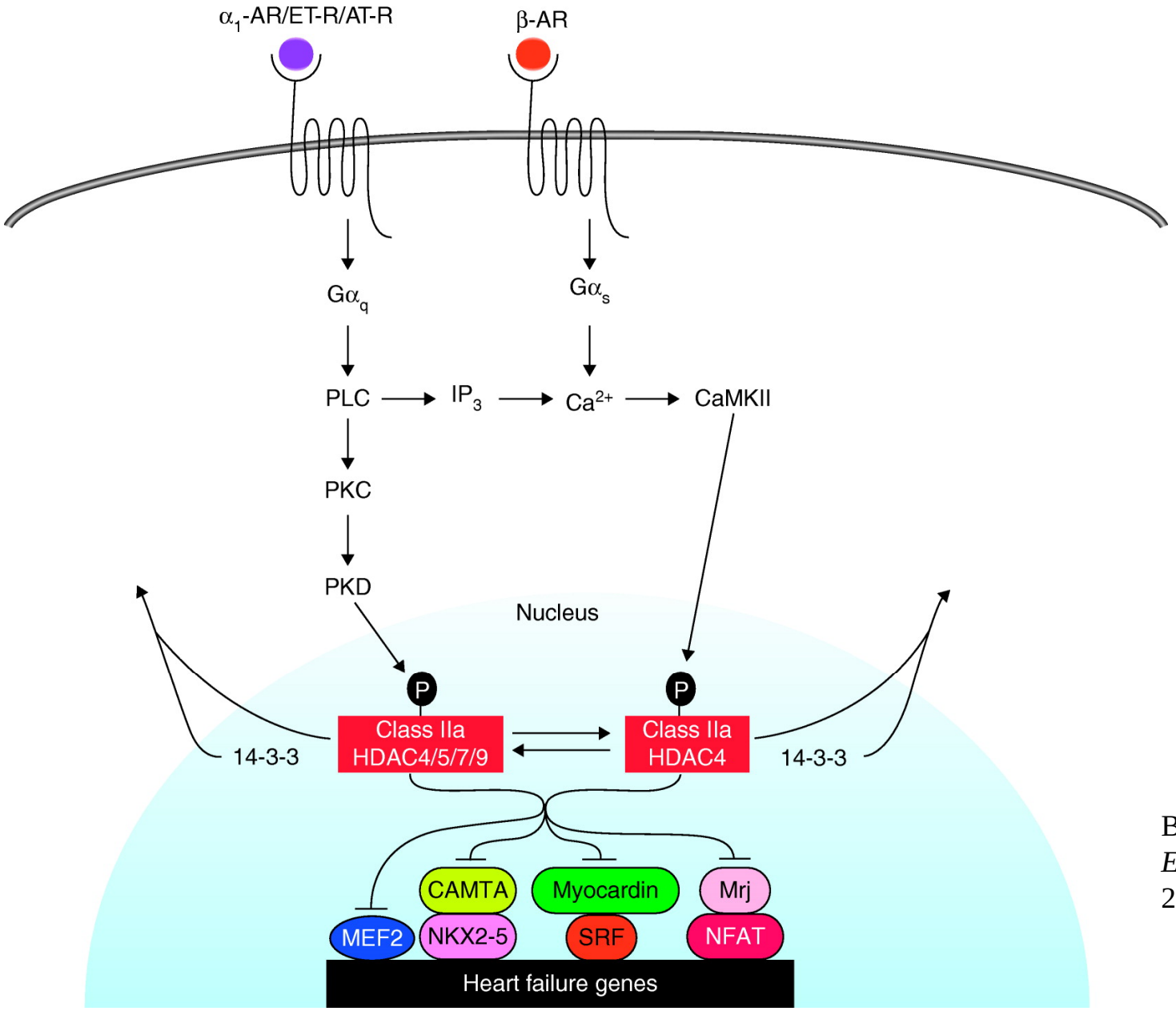
HAT (histone acetyltransferase)

p300, pCAF, Tip60

Gcn, Esa, etc

Mariadason JM. *Epigenetics* **3**:1, 28-37, 2008

Class II HDAC in cardiac hypertrophy



Bush & McKinsey
Expert Opin Ther Targets
2009

Paradoxical Effects of HDAC inhibitors:

Kook et al., *J Clin Invest* 2003

Class I HDAC inhibitor blocks TAC-induced cardiac hypertrophy

Kee et al., *Circulation*, 2006

Activation of HDAC2 by HSP70

Kee & Eom et al., *Circ Res* 2008

KLF4 is a downstream of HDAC2

Kee & Kook, *J Mol Cell Cardiol* 2009

CK2α1-induced Hdac2 S394 phosphorylation & activation

Eom et al., *Circulation*, 2011

Class II: anti-hypertrophic *vs* Class I: pro-hypertrophic

Kee & Kook, *J Biomed Biotechnol* 2011

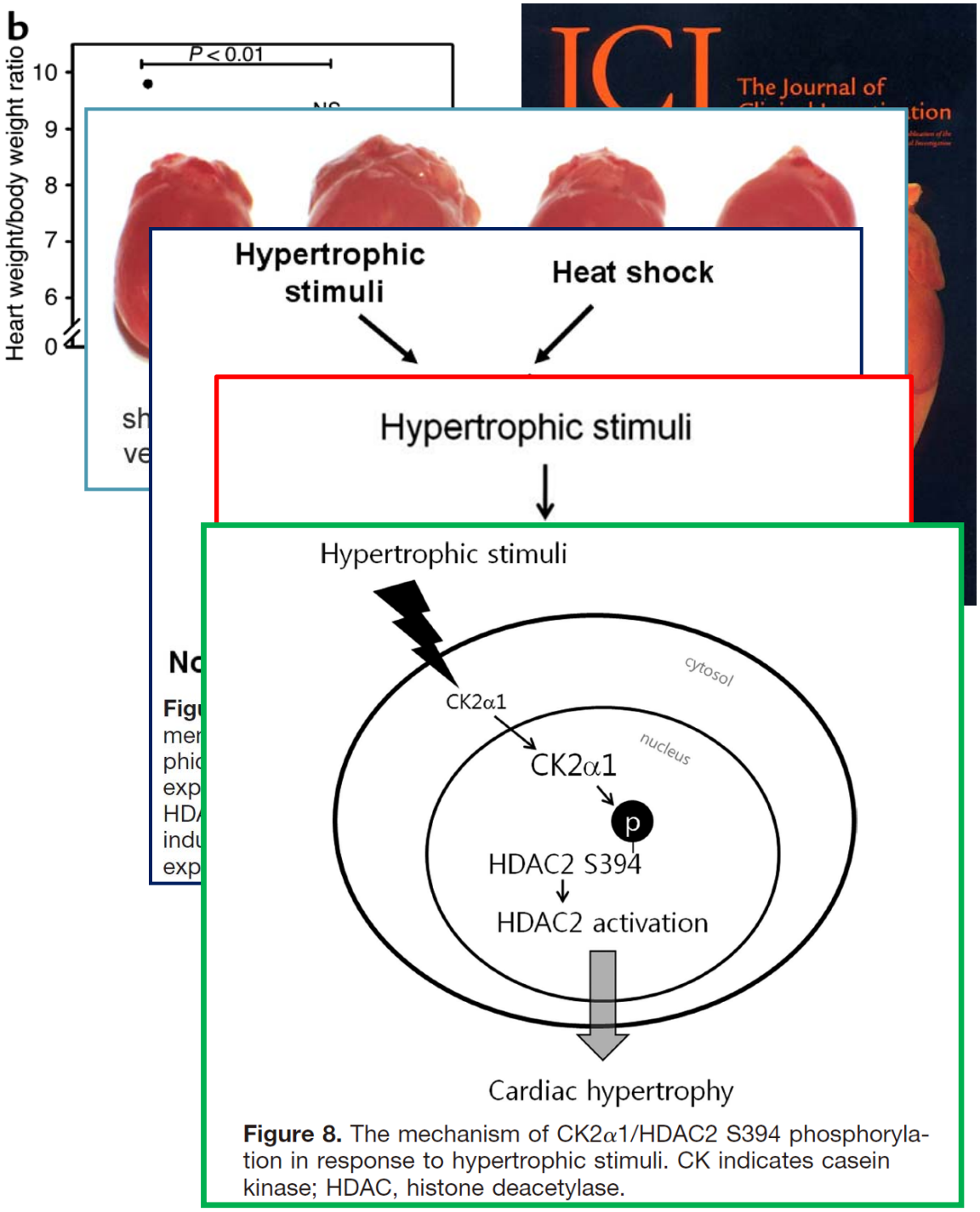
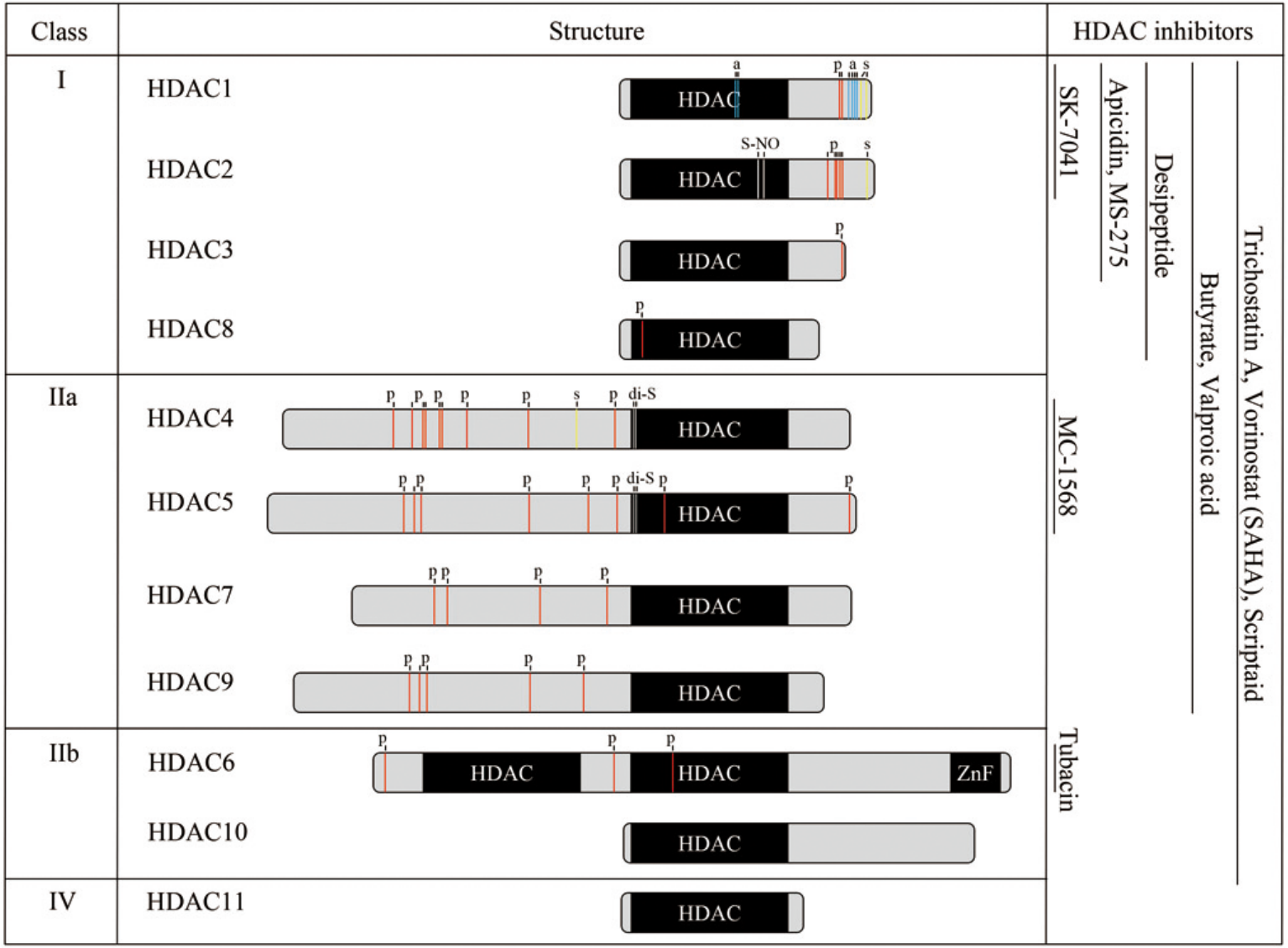


Figure 8. The mechanism of CK2α1/HDAC2 S394 phosphorylation in response to hypertrophic stimuli. CK indicates casein kinase; HDAC, histone deacetylase.

PTM of HDACs and their implications in CV diseases



Eom & Kook, *Pharmacol & Ther* 2014

Part 1

Regulation of acetylation of HDAC2 by pCAF/HDAC5 in cardiac hypertrophy

HDAC2 K75 acetylation activates HDAC2 in cardiac hypertrophy

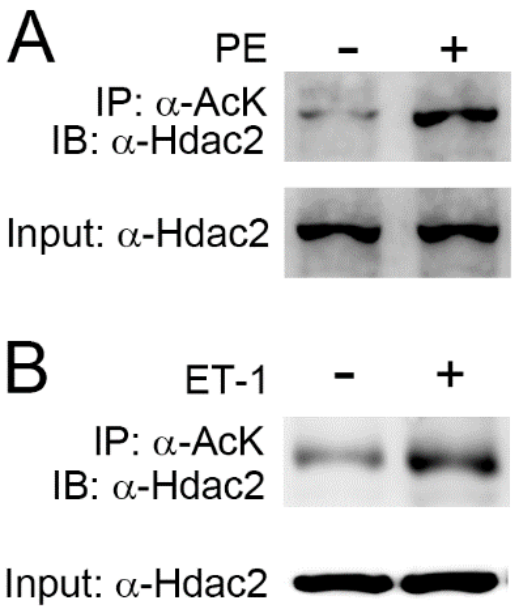
Hypertrophic stresses acetylate HDAC2

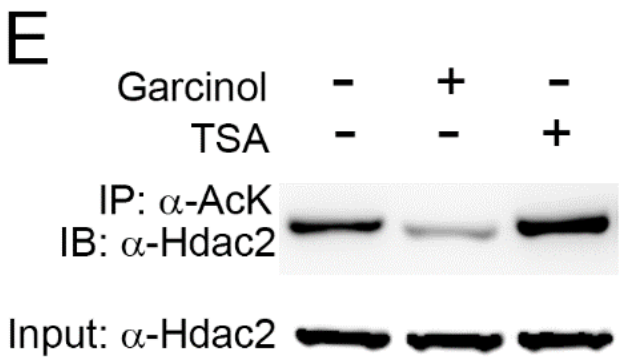
HDAC2 acetylation results in HDAC2 activation

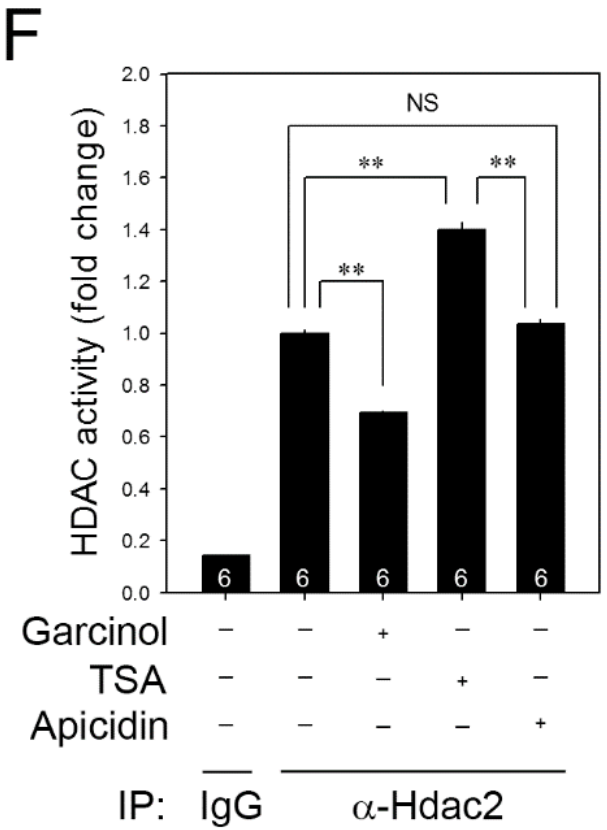
Garcinol: HAT inhibitor, acetylation ↓

TSA: class I & class II HDAC inhibitor, acetylation ↑

Apicidin: class I HDAC inhibitor, acetylation ↑



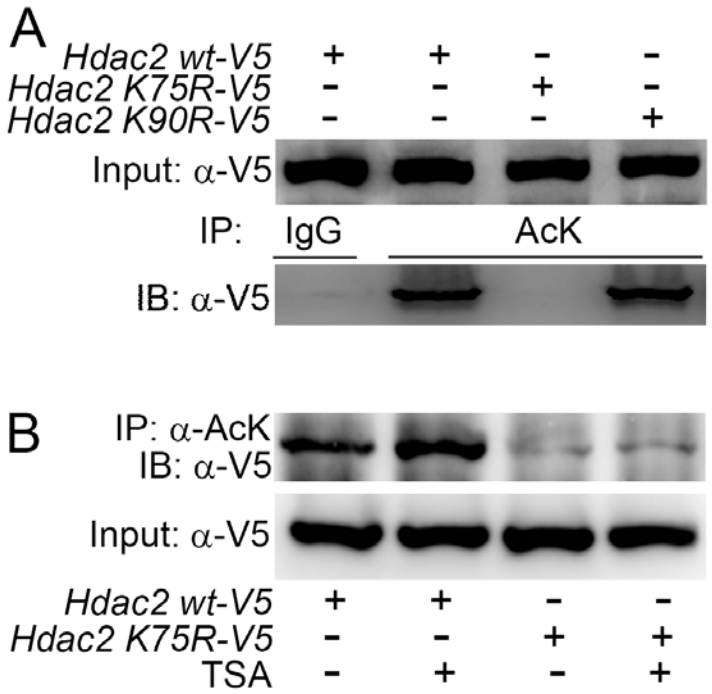


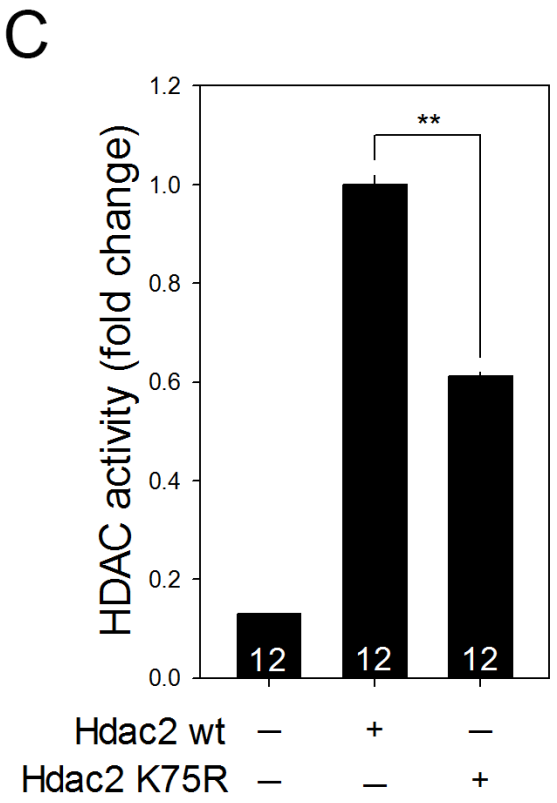


IP-based acetylation assay

Hdac2 activity

HDAC2 K75 is an acetylation target and is required for HDAC2 activation





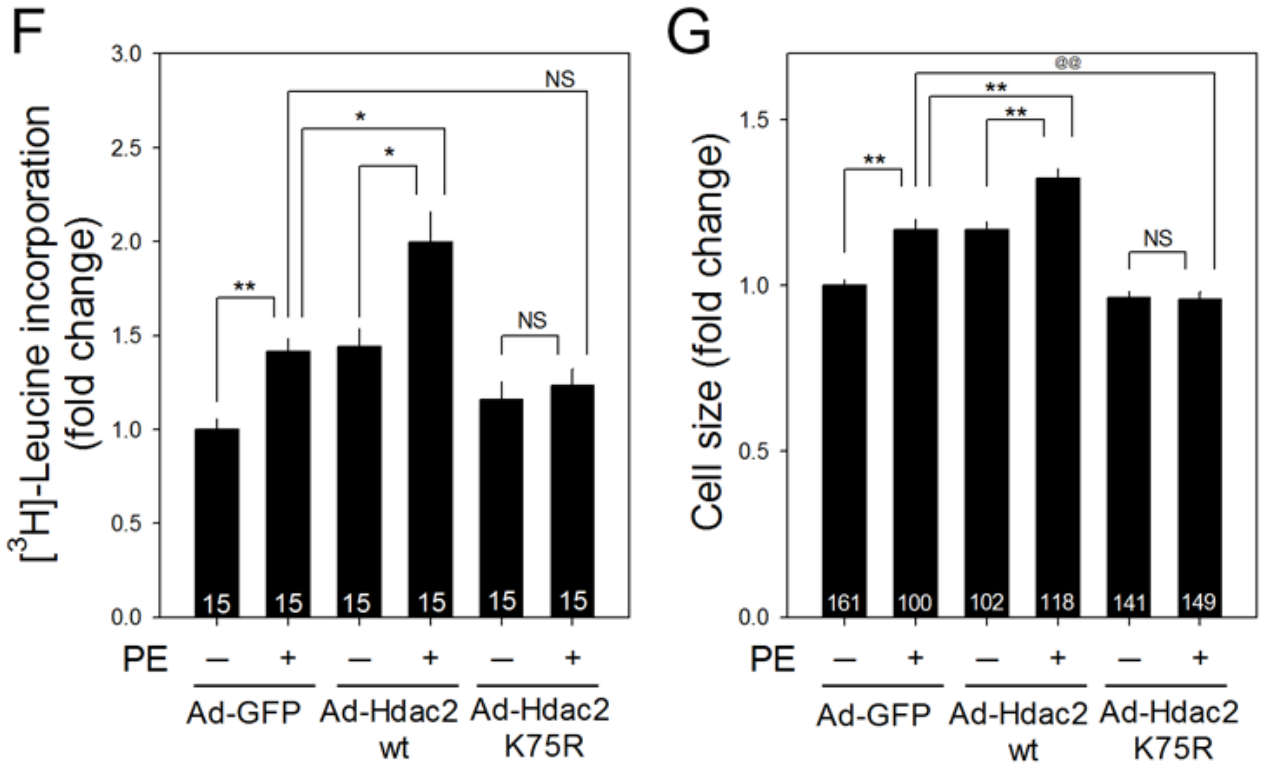
Hdac2 K75R: *by site-directed mutagenesis*

acetylation-resistant mutant

IP-based acetylation assay

HDAC activity assay

HDAC2 K75R blunts agonist-induced hypertrophy



Leucine incorporation (protein synthesis) assay

Direct cardiomyocyte size measurement after fluorescent ICC

hypertrophic stress

↓

K75 acetylation

↓

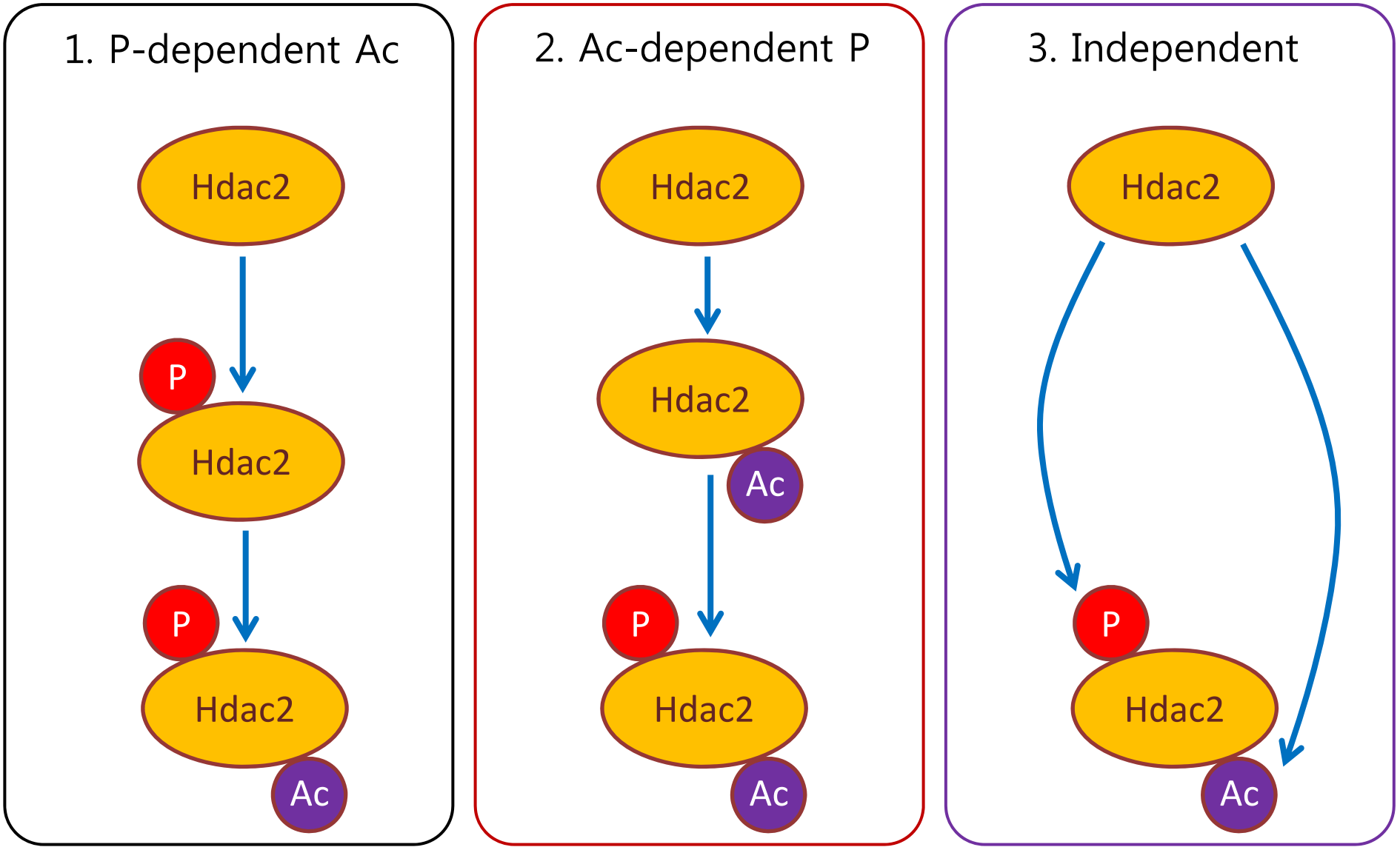
HDAC2 activity↑

↓

Hypertrophic phenotypes↑

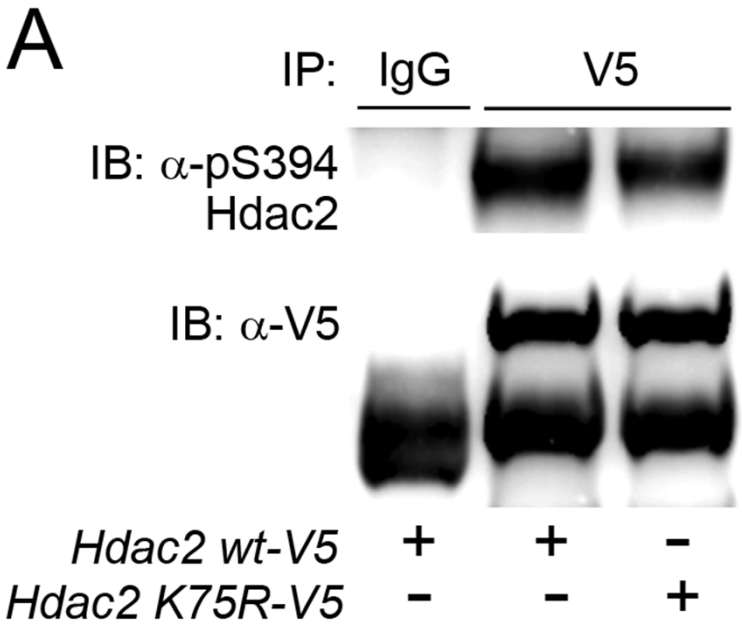
HDAC2 K75 acetylation induces HDAC2 S394 phosphorylation

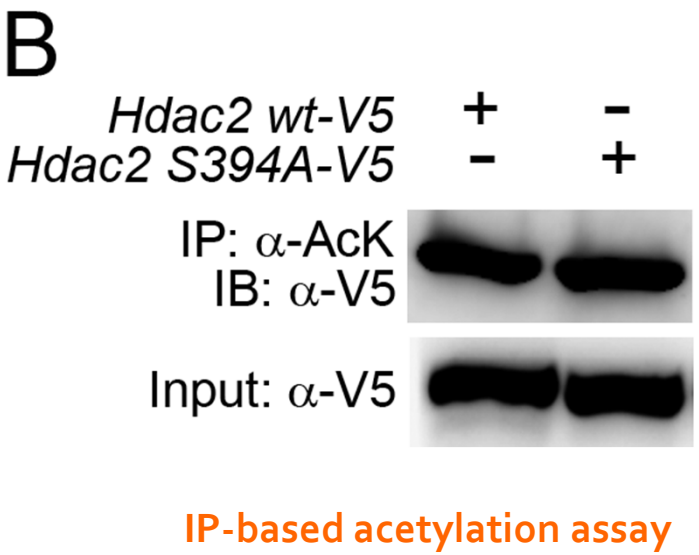
Hypothesis: acetylation vs phosphorylation



HDAC2 phosphorylation is reduced in K75R

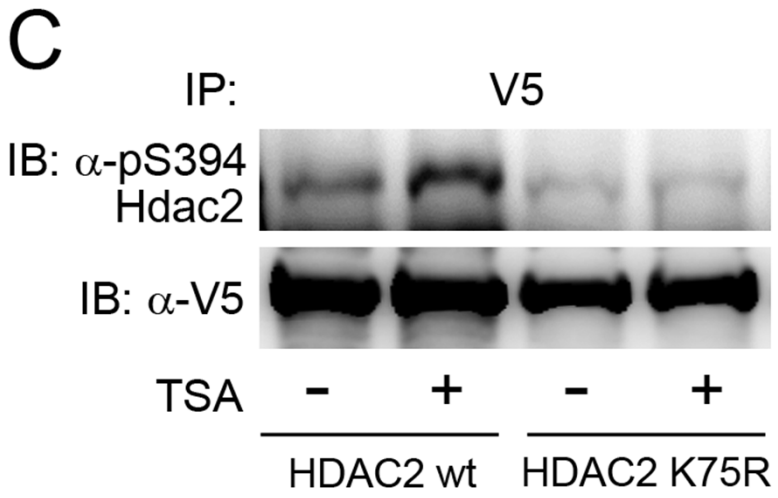
HDAC2 acetylation is NOT affected by S394A





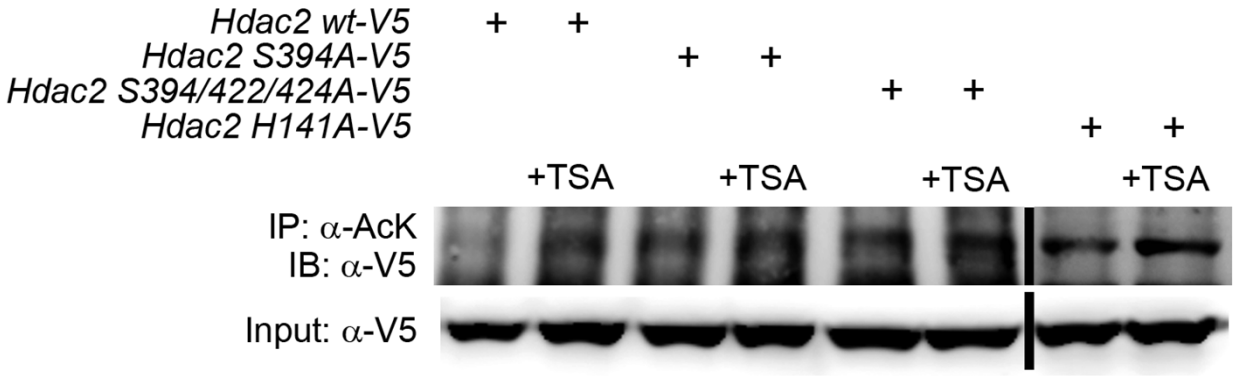
IP-based acetylation assay

HDAC2 acetylation induces S394 phosphorylation



Phosphorylation assay with pS394-specific Ab

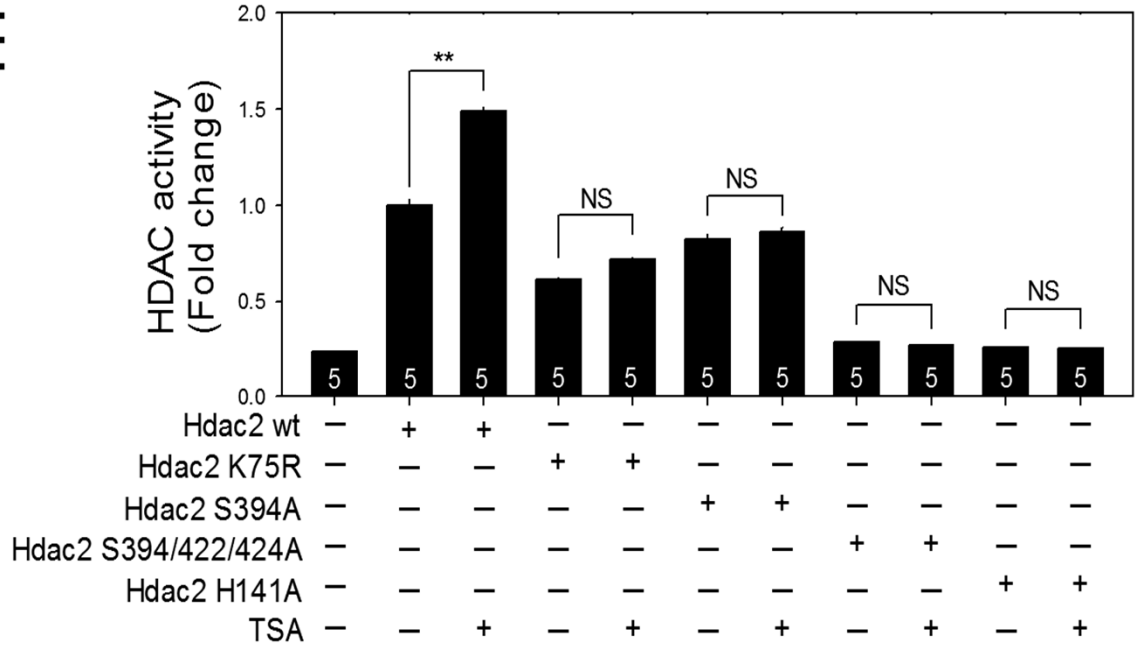
Phospho-dead HDAC2 still could be acetylated



IP-based acetylation assay

but,,, HDAC2 phosphorylation is required in acetylation-mediated HDAC2 activation

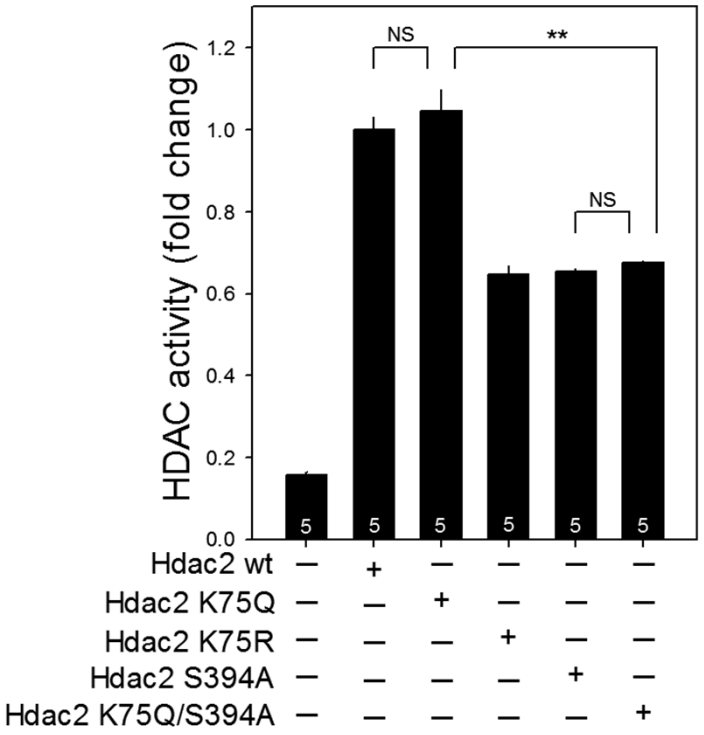
E

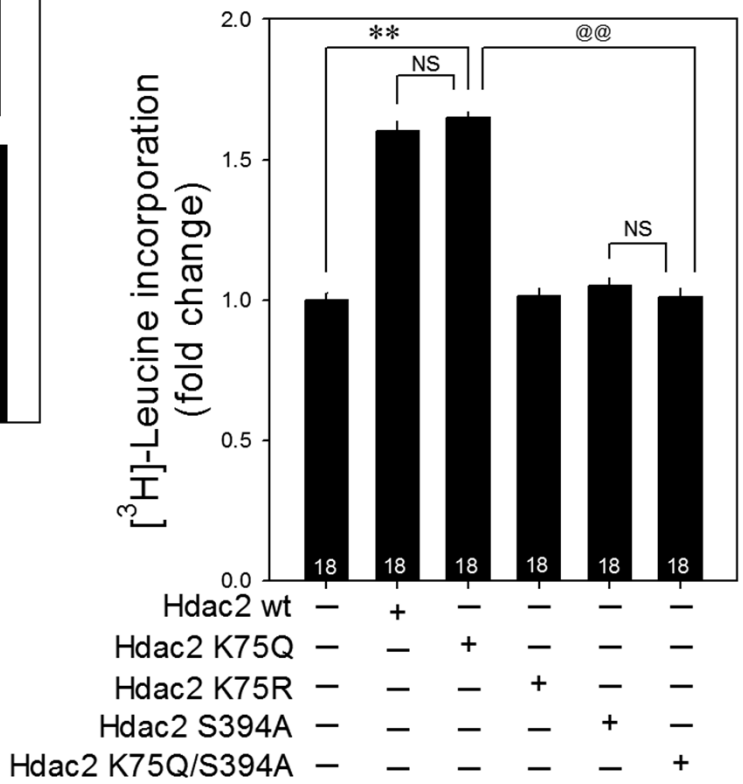


HDAC2 activity

Hdac2 K75R: acetylation-resistant
Hdac2 S394A: hypertrophy-associated phosphoylation-resistant
Hdac2 S394/422/424A: all phospho-resistant
Hdac2 H141A: enzyme-activity-dead, *by disruption of pocket structure*

HDAC2 S394 phosphorylation is required in acetylation-mediated HDAC2 activation & cardiac hypertrophy





hypertrophic stress

↓

K75 acetylation

↓

S394 phosphorylation↑

↓

HDAC2 activity ↑

↓

Hypertrophic phenotypes↑

Hdac2 K75R: acetylation-resistant
Hdac2 K75Q: acetylation-mimic
Hdac2 S394A: phospho-resistant
Hdac2 K75Q/S394A: acetylation-mimic, but phospho-resistant

pCAF / HDAC5 regulates HDAC2 acetylation

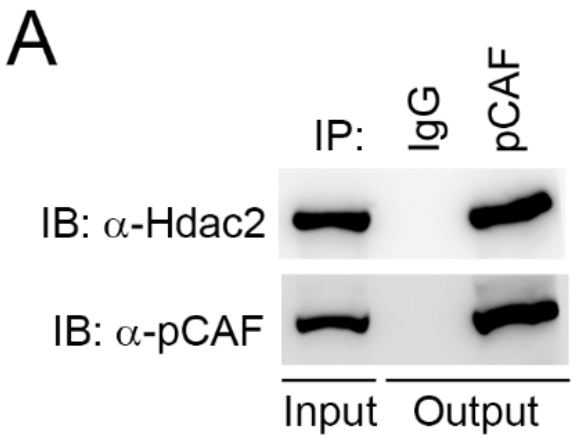
Which HAT ??? pCAF !!!

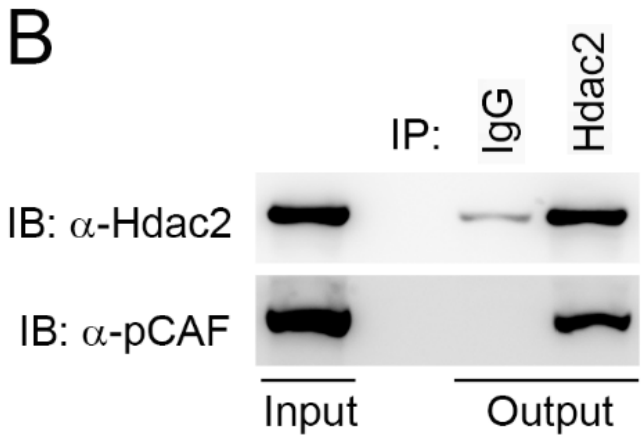
P300 does not interact with, nor acetylates HDAC2

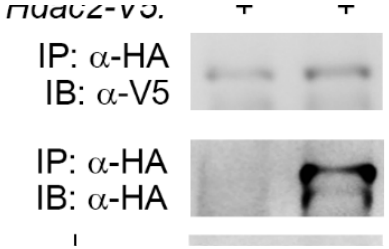
pCAF interacts with HDAC2

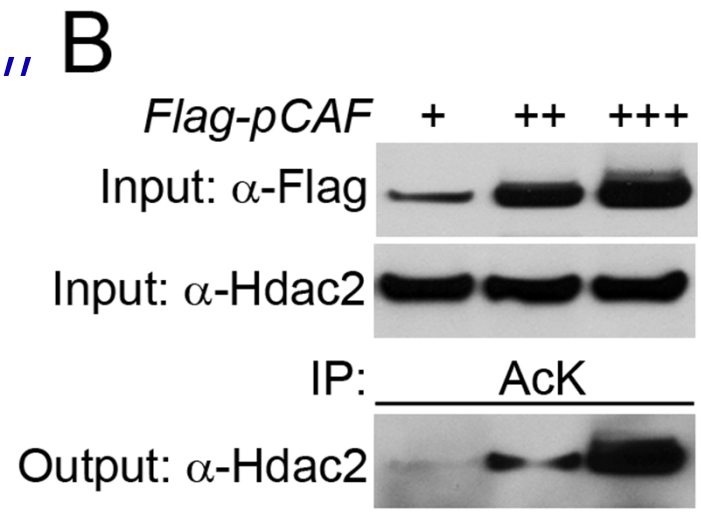
acetylates HDAC2,,,

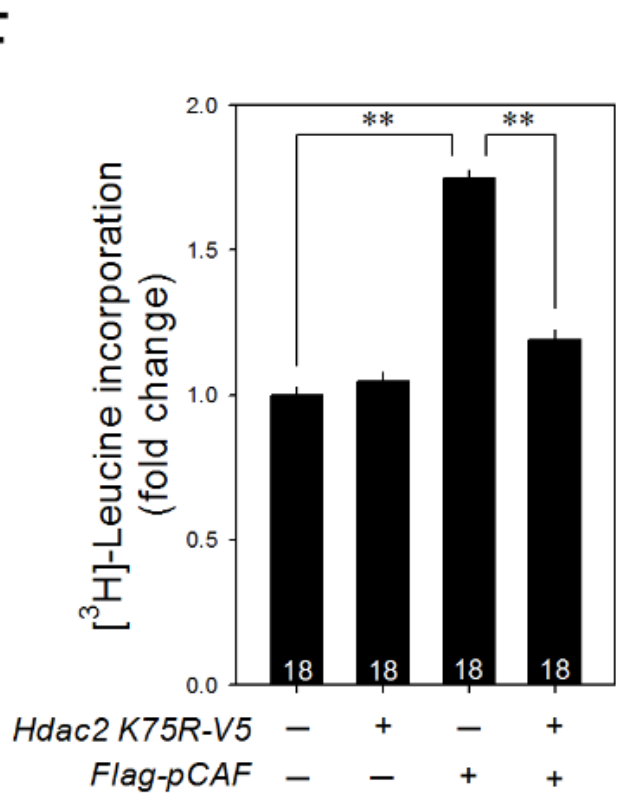
and induces cardiac hypertrophy









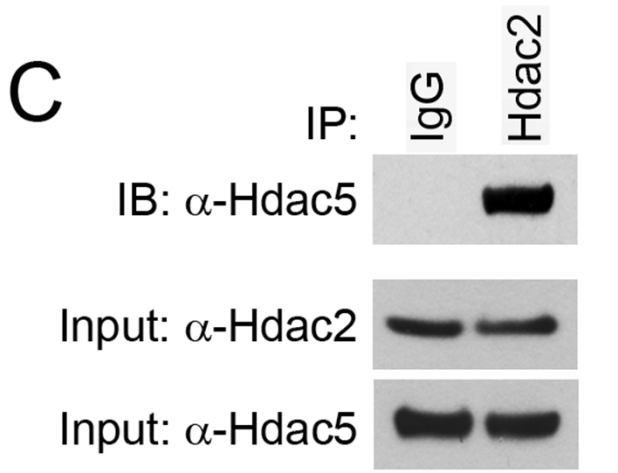


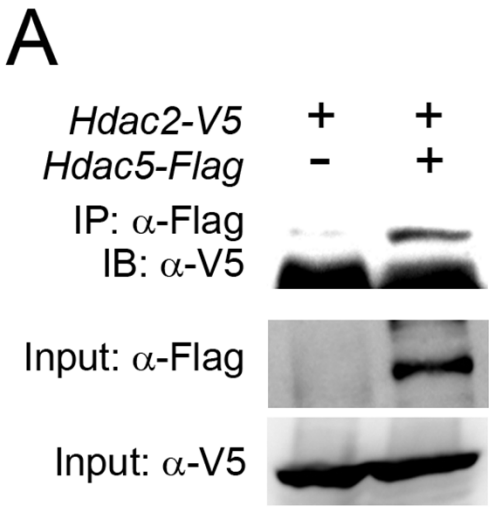
Which HDAC ??? HDAC5 !!!

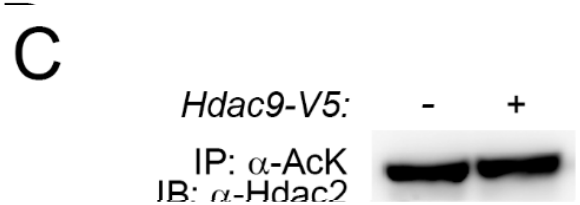
HDAC2 interacts with HDAC5,,, and with HDAC9

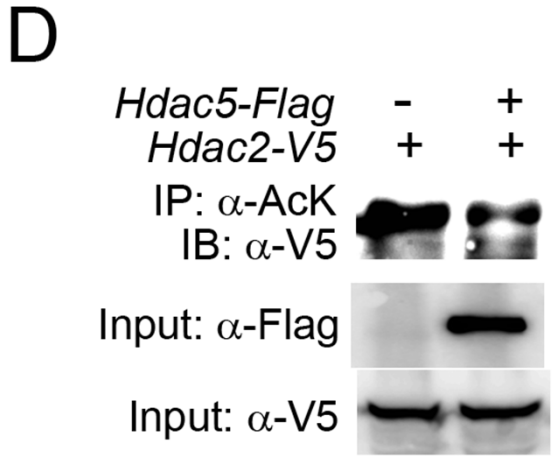
but, HDAC9 failes to deacetylate HDAC2

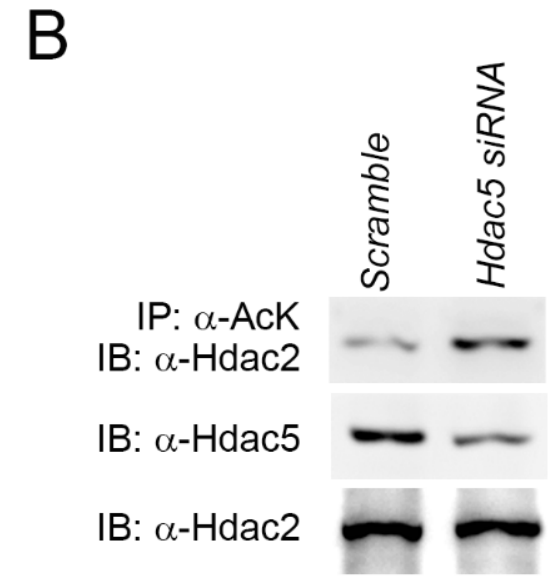
only HDAC5 deacetylates HDAC2 !!!











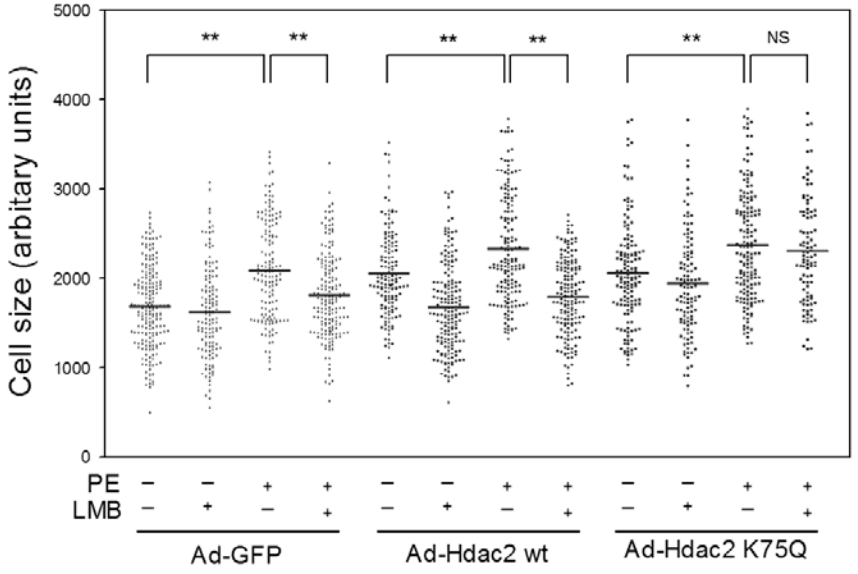
HDAC5 interplays with HDAC2 to regulate cardiac hypertrophy

LMB: enhances class II HDAC action by tethering class II HDAC in the nucleus

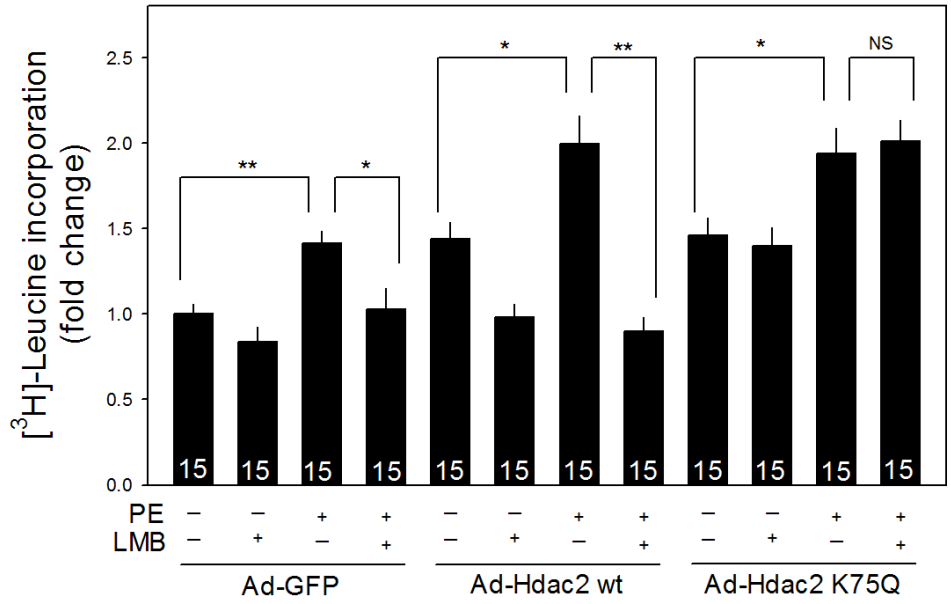
HDAC2 K75Q: acetylation-mimicking mutant

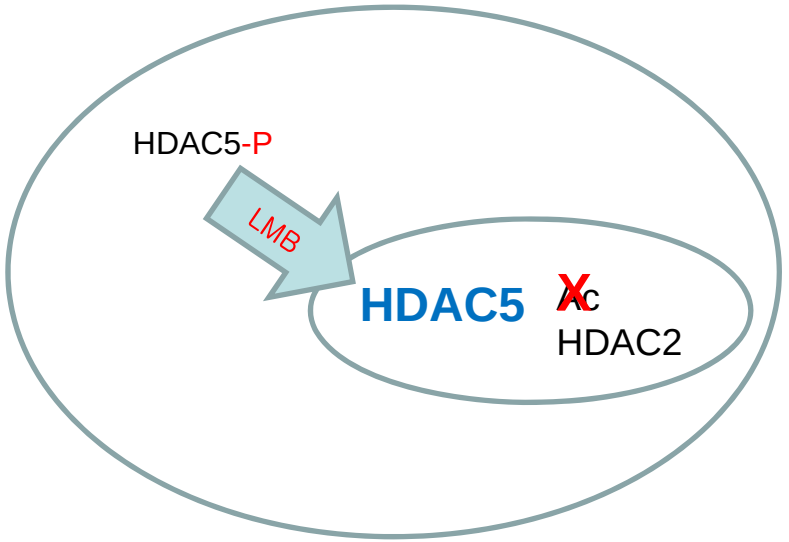
HDAC2-induced hypertrophy is blocked by enhancing HDAC5 action

A



B





HDAC2
cardiac
HDAC5

Increase in H
acetylation &
phosphoryla
HDAC5 knoc

Tail vein inje
adeno-HDA
HDAC5 knoc

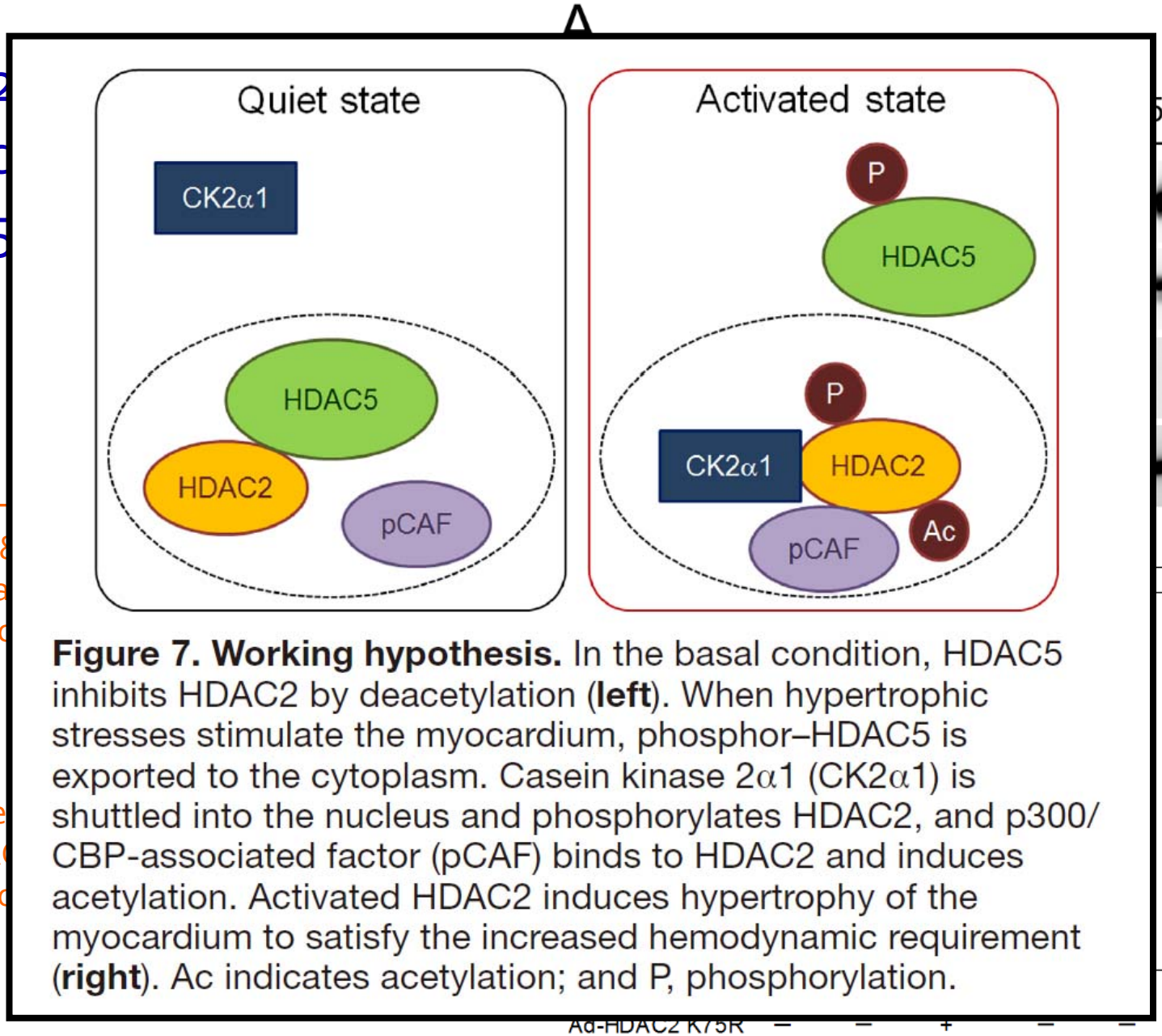
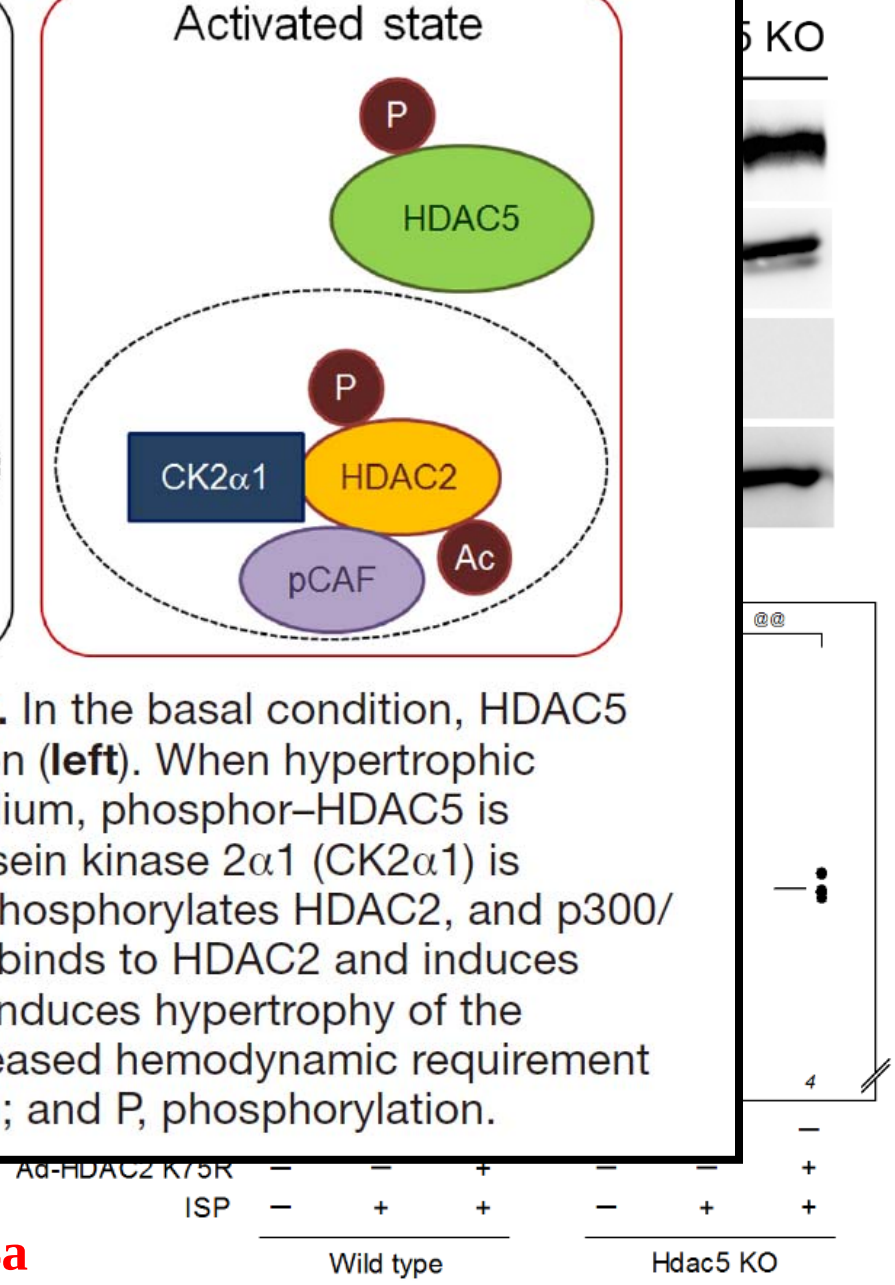


Figure 7. Working hypothesis. In the basal condition, HDAC5 inhibits HDAC2 by deacetylation (**left**). When hypertrophic stresses stimulate the myocardium, phosphor–HDAC5 is exported to the cytoplasm. Casein kinase 2α1 (CK2α1) is shuttled into the nucleus and phosphorylates HDAC2, and p300/CBP-associated factor (pCAF) binds to HDAC2 and induces acetylation. Activated HDAC2 induces hypertrophy of the myocardium to satisfy the increased hemodynamic requirement (**right**). Ac indicates acetylation; and P, phosphorylation.



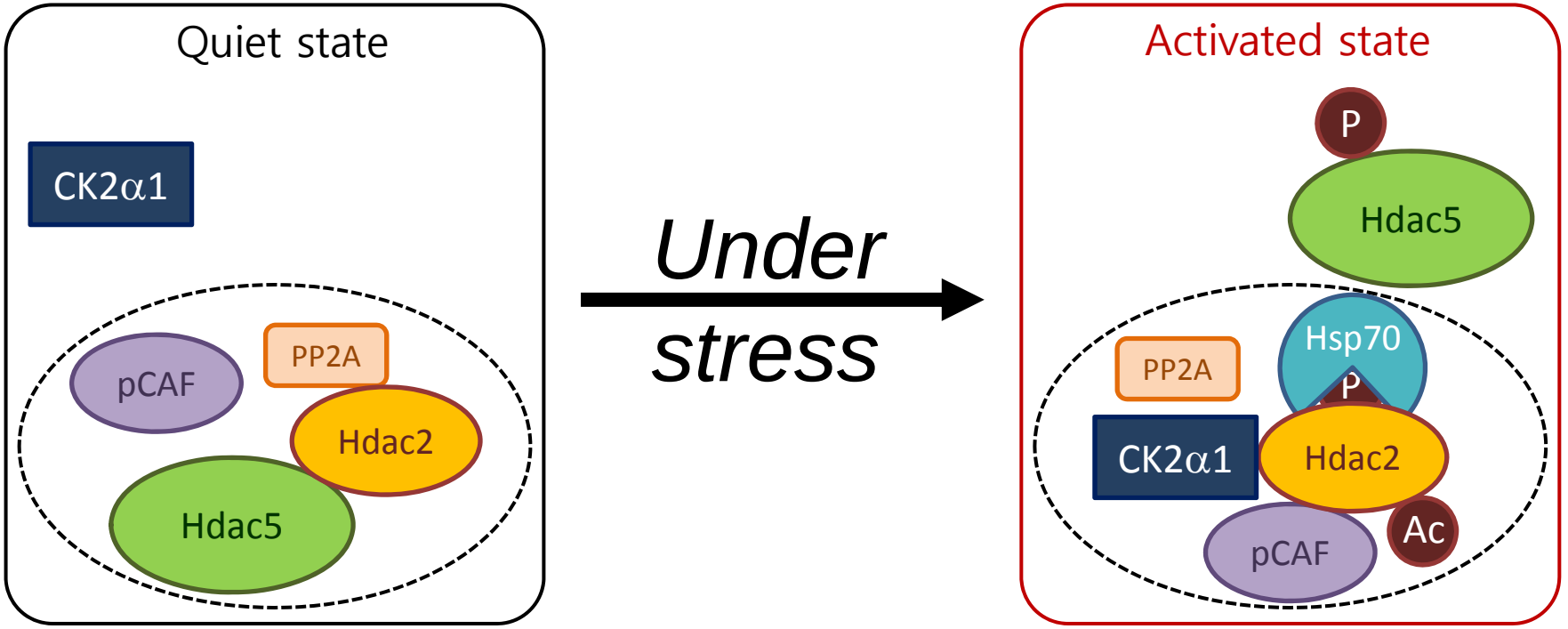
Eom et al., *Circ Res* 114:1133-1143, 2014a

Part 2

PP2A/HSP70 dynamically regulates HDAC2 phosphorylation

Hypothesis

Phosphorylation regulation mechanism other than CK2α1?

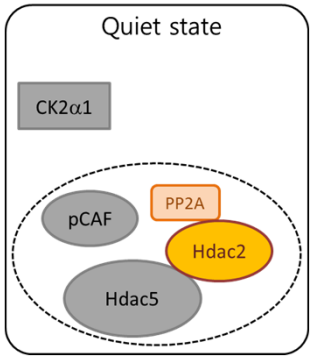


Eom and Kook. *BMB report* **48**: 131-138, 2015

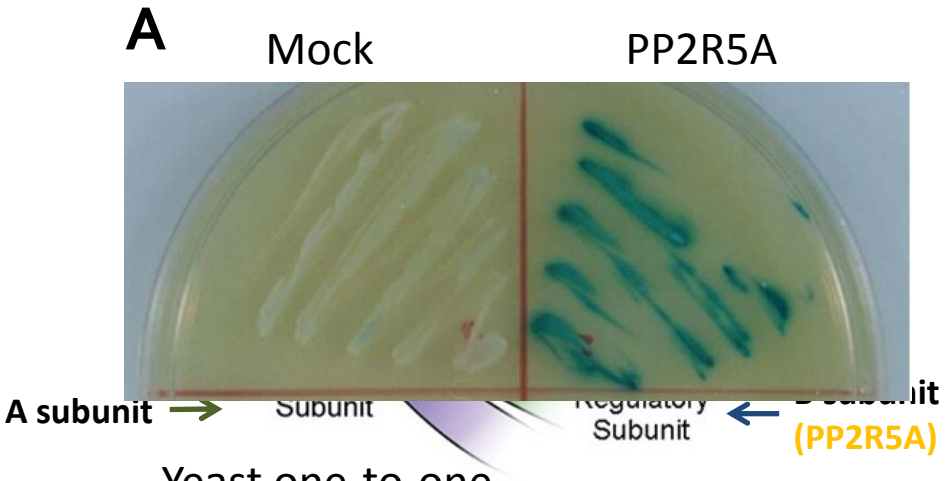
Protein phosphatase 2A attenuates hypertrophy by dephosphorylation of HDAC2

PP2A binds to and dephosphorylates HDAC2

PP2A inhibits HDAC2 activity



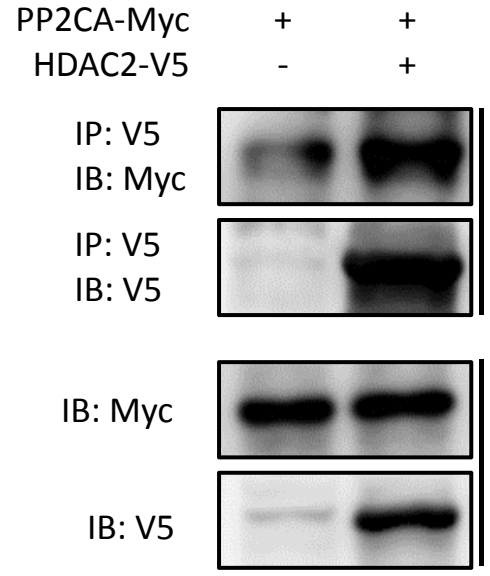
A



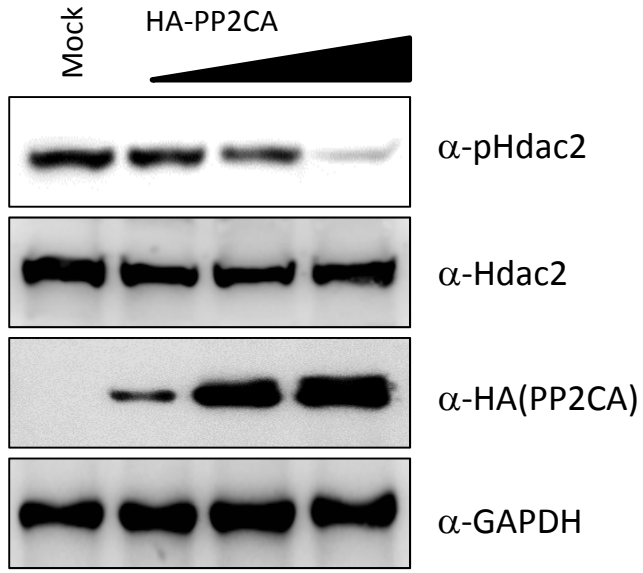
Yeast one-to-one

pGBKT7-HDAC2 / pGADT7-PP2R5A

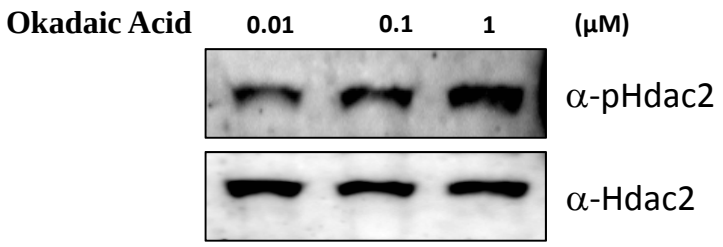
B



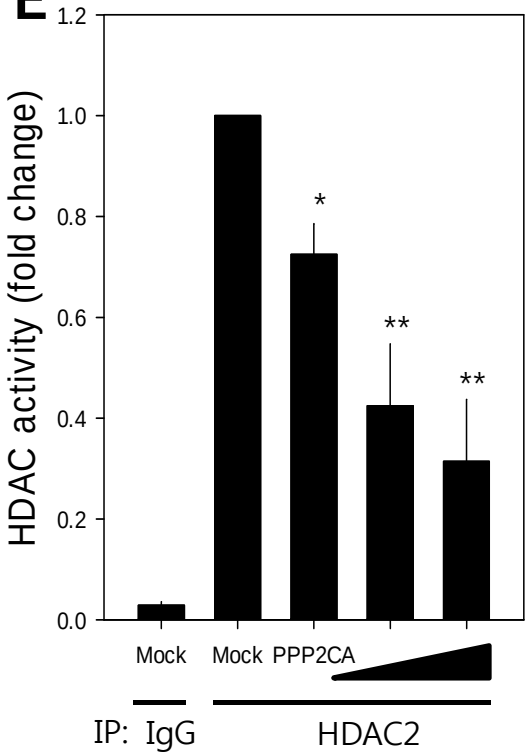
C



D



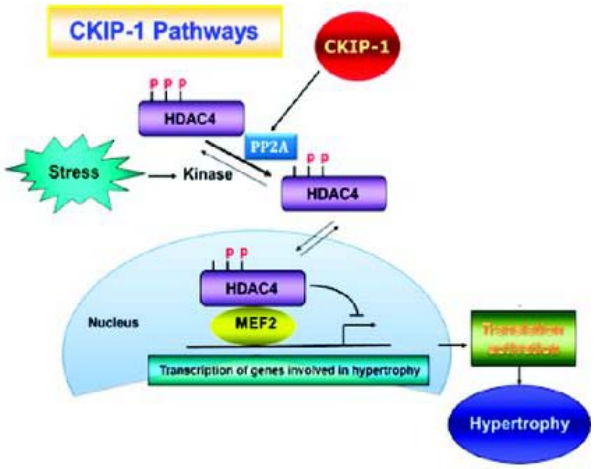
E



PP2CA inhibits cardiac hypertrophy

by dephosphorylating HDAC4 (Ling et al., 2012)

by dephosphorylating HDAC2 ?

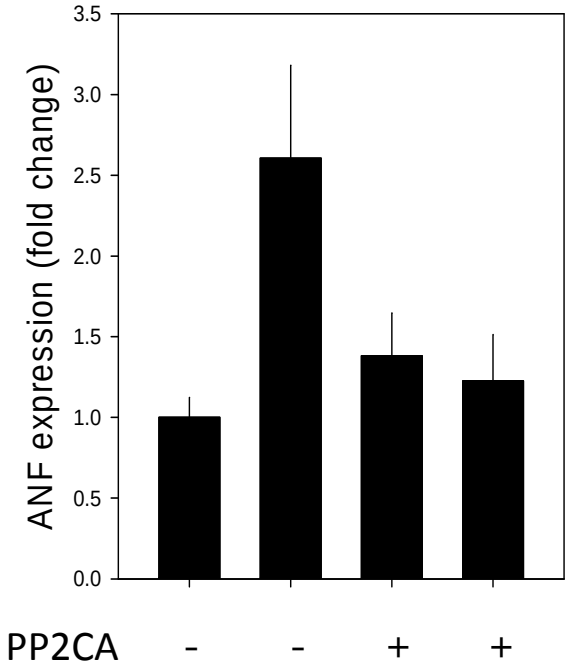


CKIP-1 Inhibits Cardiac Hypertrophy by Regulating Class II Histone Deacetylase Phosphorylation Through Recruiting PP2A

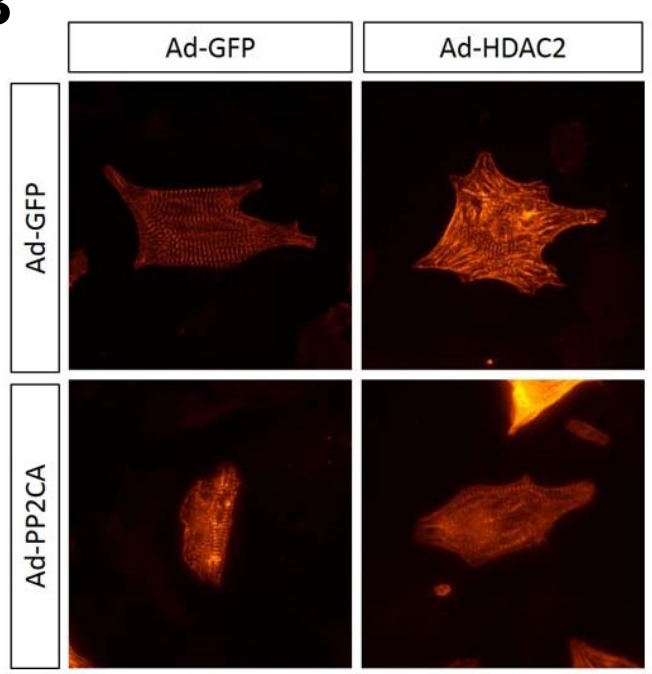
Shukuan Ling, PhD*; Qiao Sun, MD, PhD*; Yuheng Li, MA; Luo Zhang, MD; Pengfei Zhang, MA; Xiaogang Wang, MD; Chunyan Tian, MD, PhD; Qi Li, MA; Jinping Song, MD; Hongju Liu, BA; Guanghan Kan, MA; Hongqing Cao, MA; Zengming Huang, BA; Jielin Nie, BA; Yanqiang Bai, MD; Shanguang Chen, PhD; Yinghui Li, PhD; Fuchu He, PhD; Lingqiang Zhang, PhD; Yingxian Li, PhD

Ling et al. Circulation. 2012; 126:3 028-3040

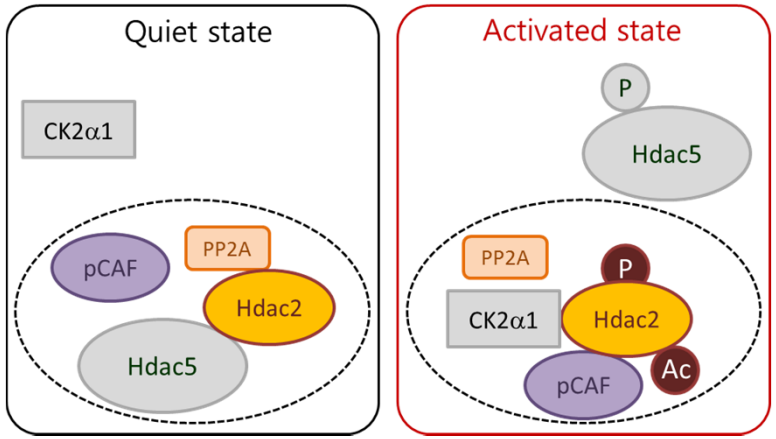
A

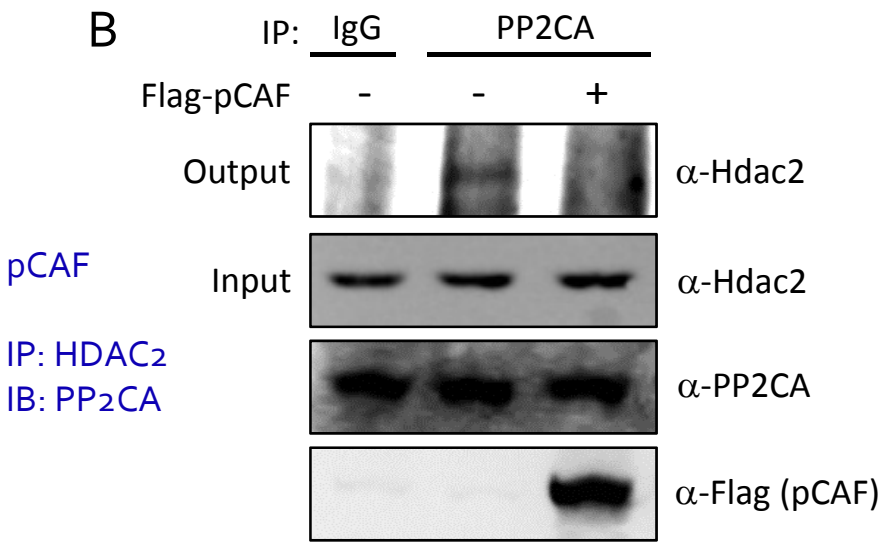


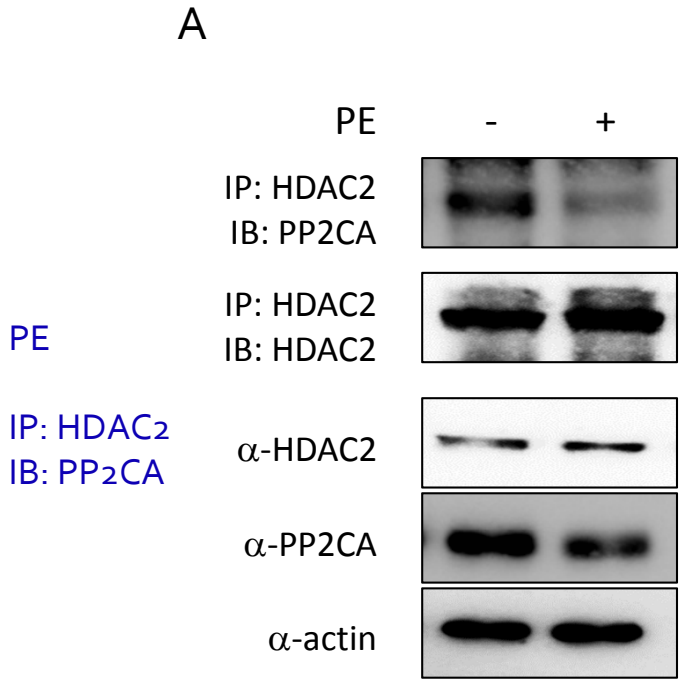
B

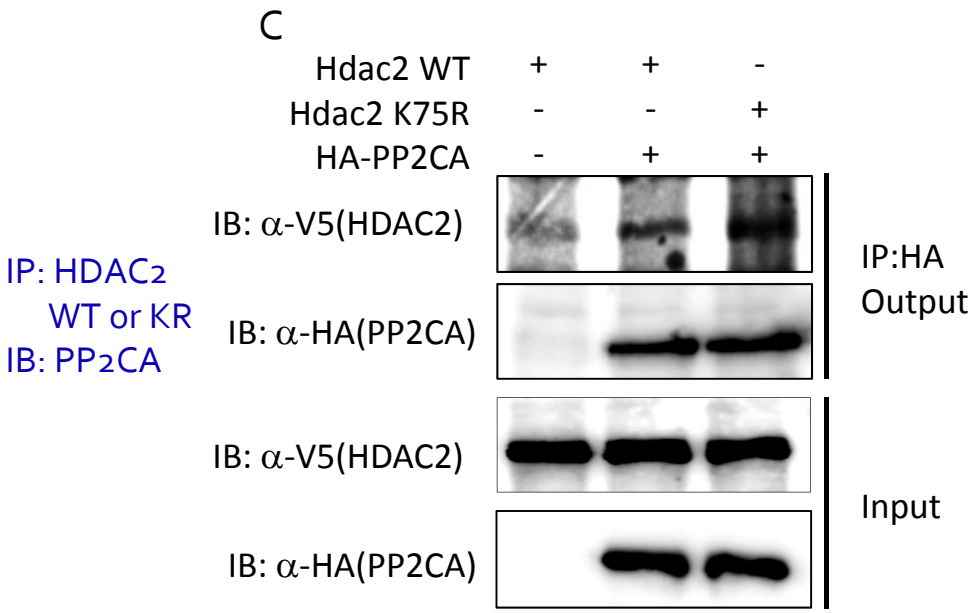


PP2CA dissociates from HDAC2 by hypertrophic stress or by HDAC2 acetylation









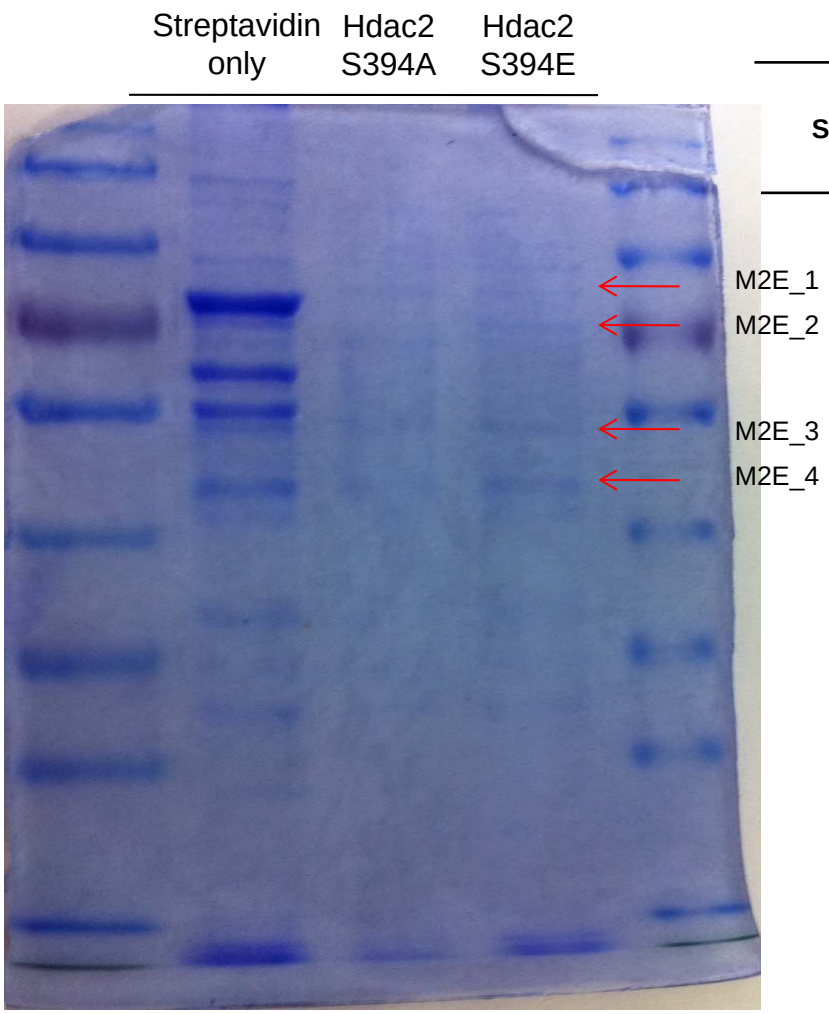
HSP70 specifically binds to phosphorylated HDAC2 to maintain its activity

Phospho-HDAC2-specific binding partners

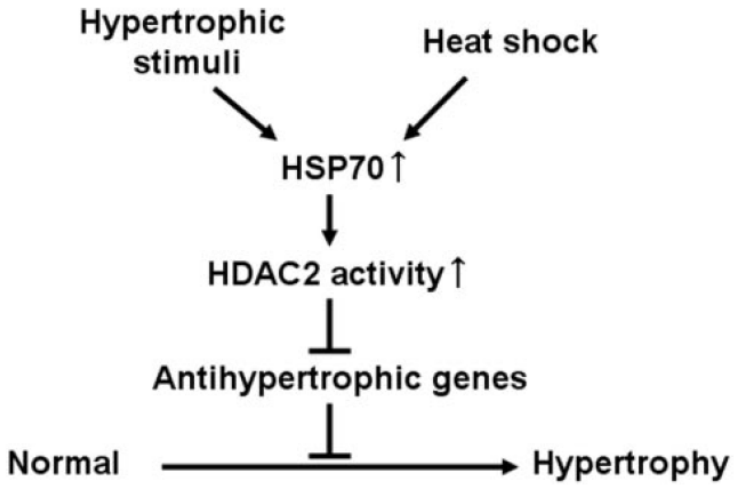
Protein-precipitation with biotinylated peptide

Bait: (biotin-conjugated) 15 amino acid-long peptide of HDAC2 flaking S394A (S->A, negative)
or S394E (S->E, mimic)

Prey: mouse heart lysates



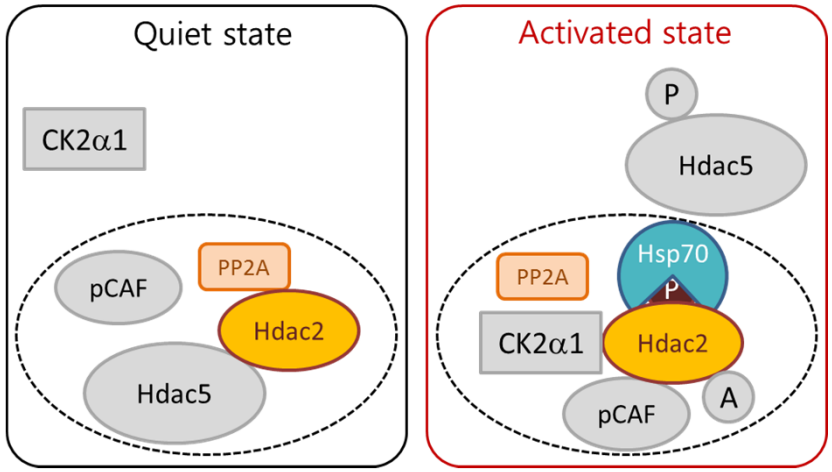
Sample	Gene		MW	Subcellular location
S394E_2.pkl	Hsp70	Inducible Heat shock protein	72 kDa	Nucleus + Cytoplasm



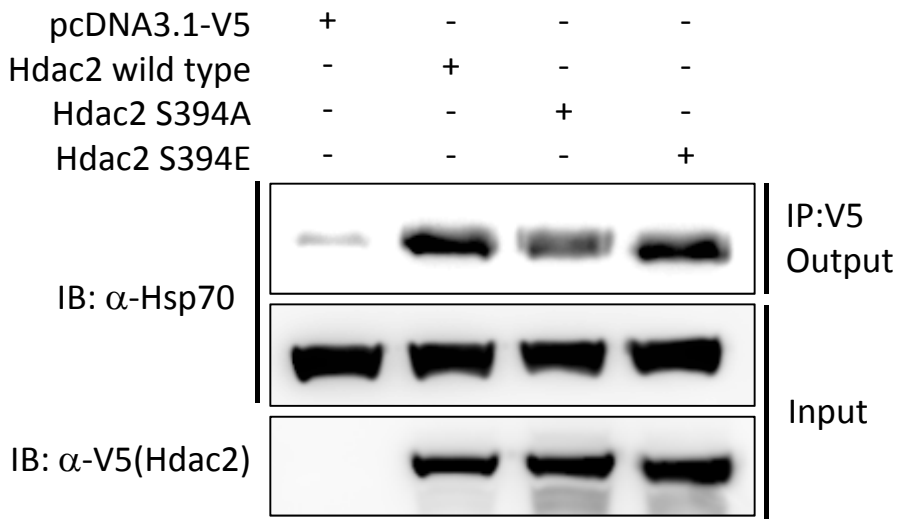
Kee & Eom et al., *Circ Res*. **103**: 1259-1269, 2008.

HSP70 preferentially binds to phosphorylated HDAC2

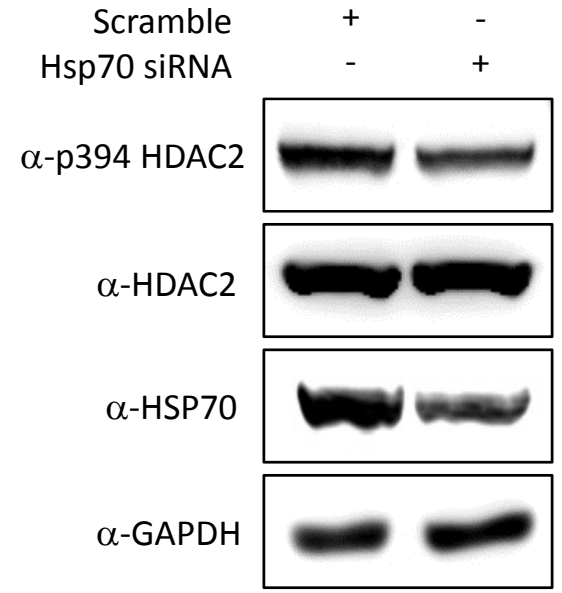
HSP70 is required for phosphorylation of HDAC2



A

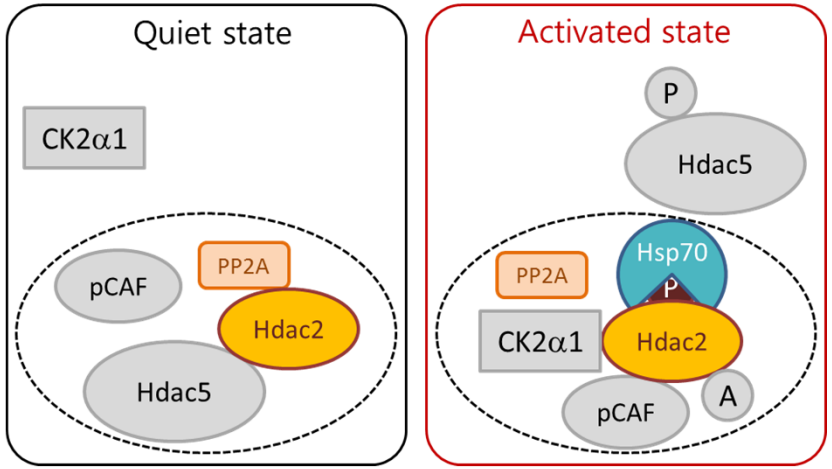


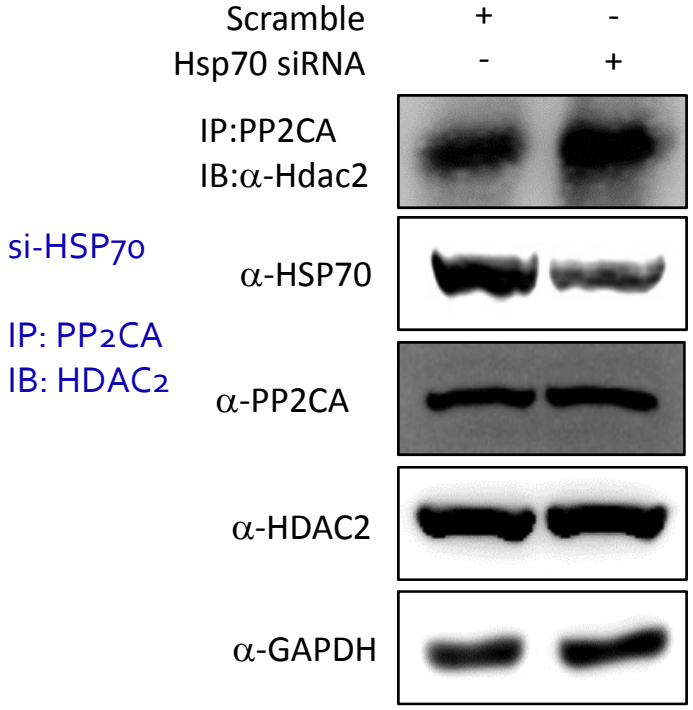
B

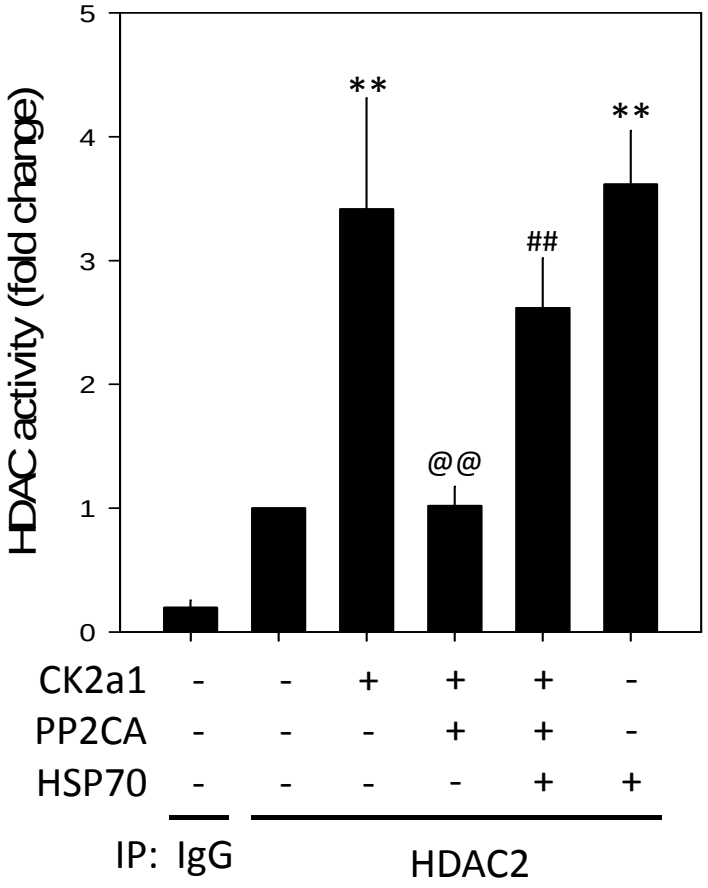


HSP70 interferes with binding of PP2CA to HDAC2

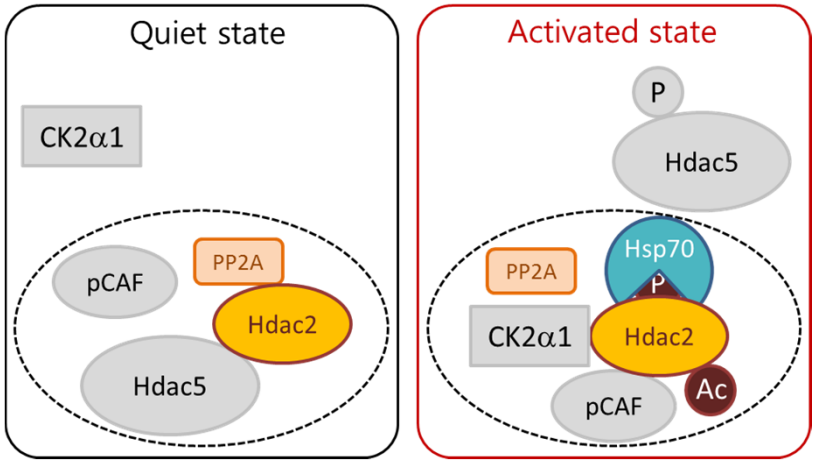
HSP70 protects HDAC2 activity from PP2CA

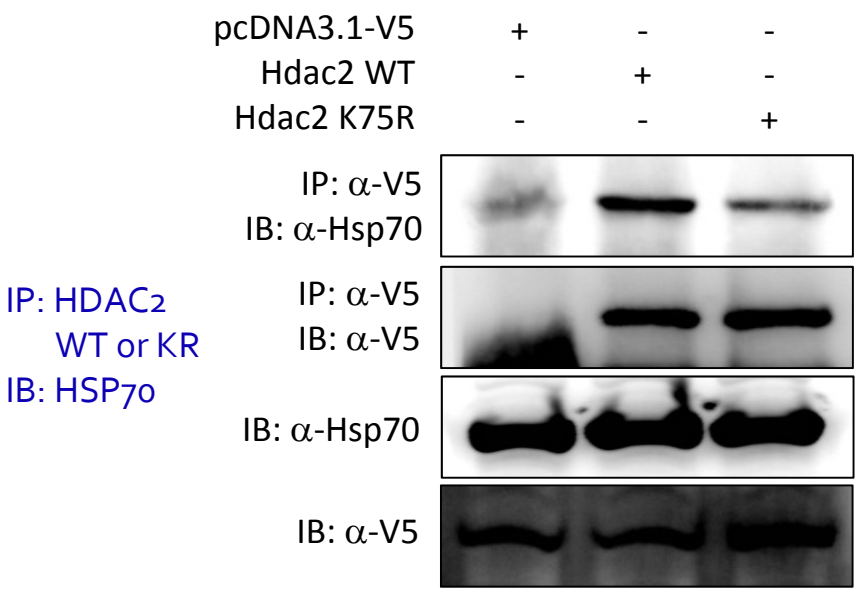






HDAC2 K75 acetylation is required for binding of HSP70 to HDAC2





hypertrophic stress

↓

K75 acetylation

↓

PP2A dissociation

↓

CK2α1 recruitment

↓

HDAC2 S394 phosphorylation↑

↓

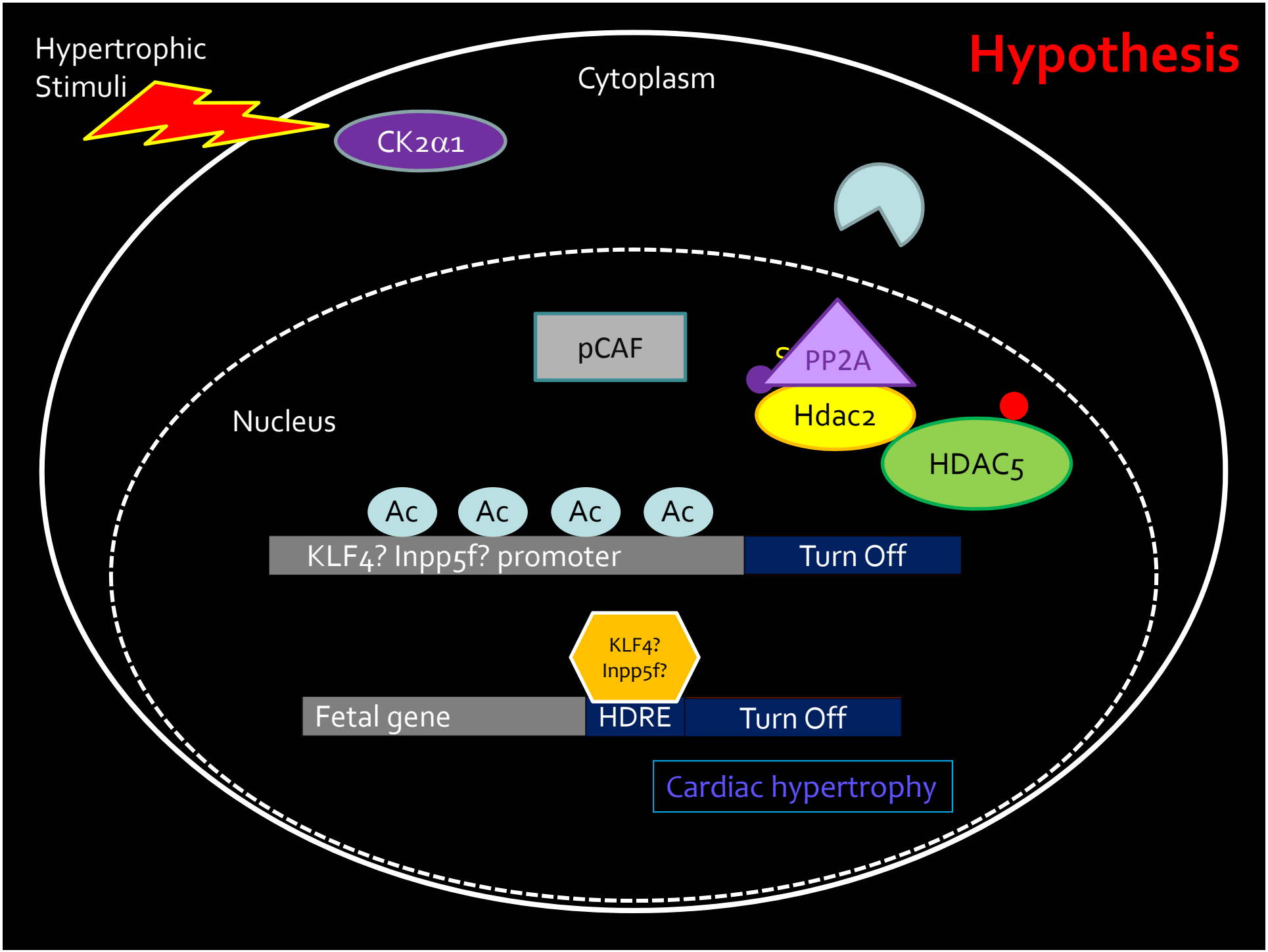
HSP70 binding to pHDAC2

↓

HDAC2 activity ↑

↓

Hypertrophic phenotypes↑


Hypertrophic Stimuli
Cytoplasm
Hypothesis
CK2α1
pCAF
PP2A
Hdac2
HDAC5
Nucleus
Ac
Ac
Ac
Ac
KLF4? Inpp5f? promoter
Turn Off
KLF4? Inpp5f?
Fetal gene
HDRE
Turn Off
Cardiac hypertrophy

Acknowledgement

KOOK Lab

Gwang Hyeon Eom, MD, PhD, Assistant Professor
Hosouk Joung, PhD
Duk-wha Kwon, PhD
Yoonseok Nam, PhD Candidate
Nakwon Choe, MS
Sera Shin, BS
Hyun-Ki Min, BS
Taewon Kook, BS
Eun Mi Kim, Lab. Manager

Young Guk Cho, animal model, echocardiogram, MD, PhD, Department of Pediatrics

Collaborators

Kwang-Il Nam, histology, MD, PhD, Department of Anatomy
Jung-Joon Min, tail vein injection, MD, PhD, Department of Nuclear Medicine

Woo-Jin Park, adult cardiomyocytes, PhD, Department of Life Science, GIST
In-Kyu Lee, adenovirus, MD, PhD, Kyungbook University

Eric Olson, HDAC5 ko animal, PhD, UT Southwestern
Rhonda Bassel-Duby, HDAC5 ko animal & data discussion, PhD, UT Southwestern
Jonathan A. Epstein, data discussion & mentor, MD, University of Pennsylvania