In vitro functional study of Cardiomyocyte

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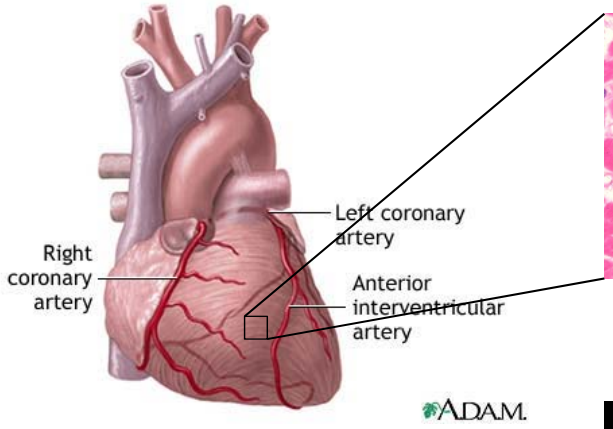
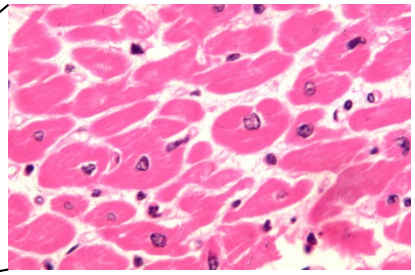
Heart and cardiomyocyte





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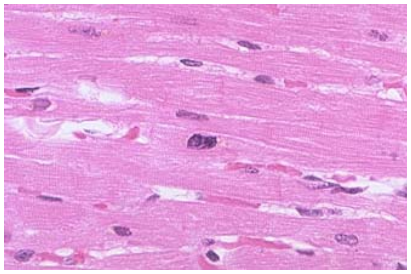
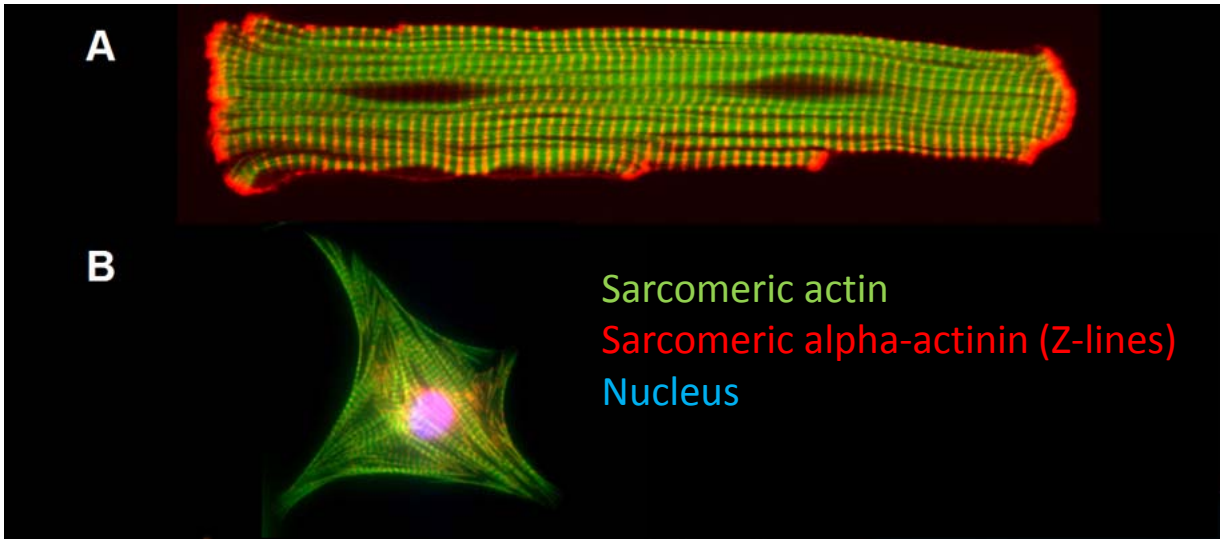
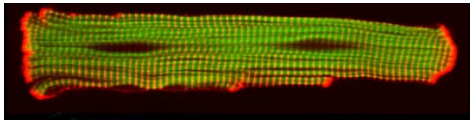




A: Adult Rat Cardiac Myocyte (nucleus not shown)
B: Neonatal Rat Cardiac Myocyte

http://diseasebiophysics.seas.harvard.edu/research/mechanotransduction/

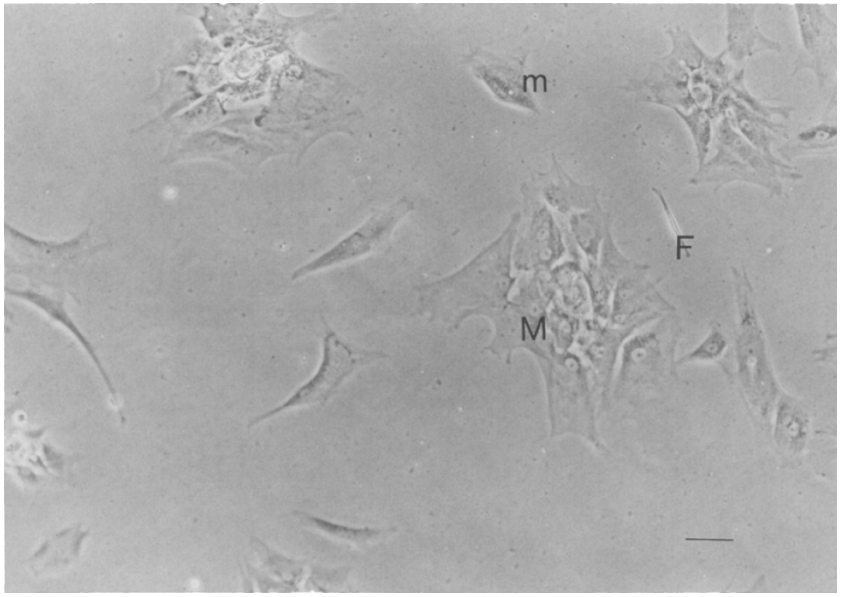
Characteristics of cardiomyocytes

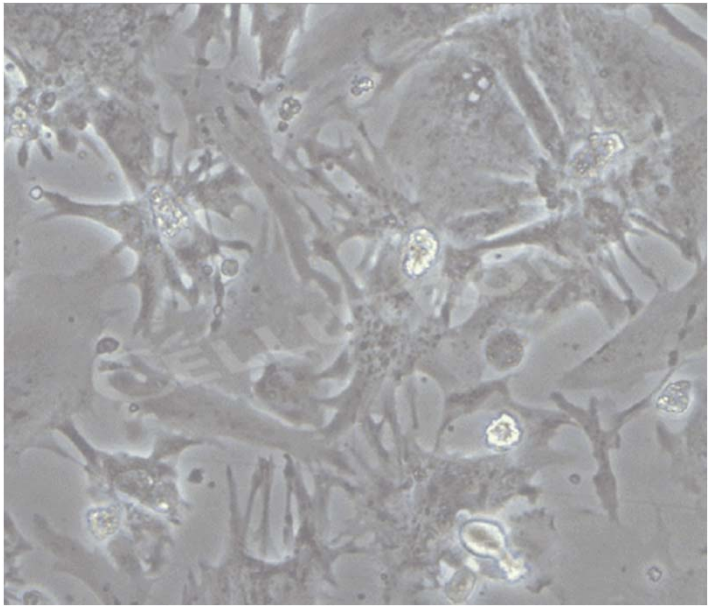


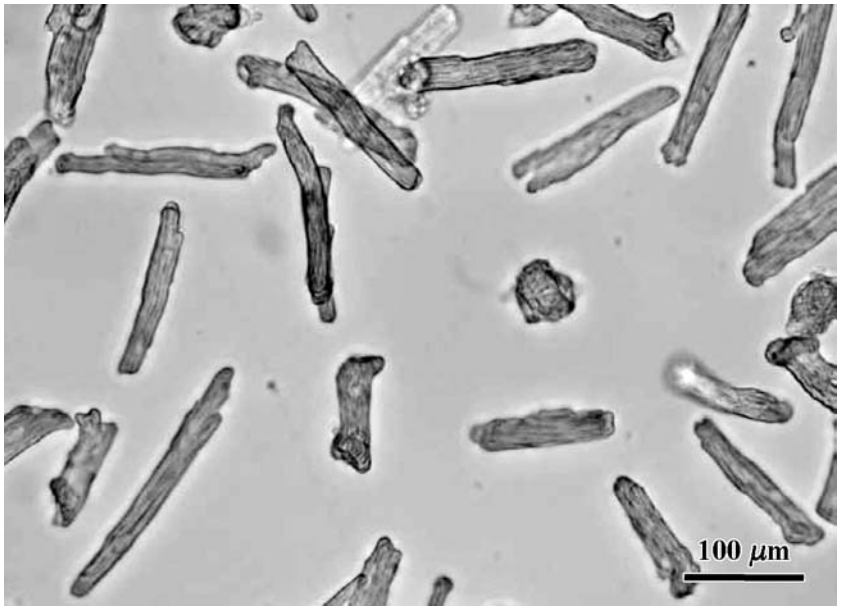
1. Involuntary striated muscle
2. Can contain one, two, or very rarely three or four cell nuclei
3. Oxygen and nutrients are supplied not by blood in the cardiac chamber directly but by coronary arteries.
4. Cardiac muscle requires extracellular calcium ions for contraction to occur
5. Until recently, it was commonly believed that cardiac muscle cells could not be regenerated. However, a study reported in the April 3, 2009 issue of Science contradicts that belief. Olaf Bergmann and his colleagues at the Karolinska Institute in Stockholm tested samples of heart muscle from people born before 1955 when nuclear bomb testing caused elevated levels of radioactive carbon 14 in the Earth's atmosphere. They found that samples from people born before 1955 did have elevated carbon 14 in their heart muscle cell DNA, indicating that the cells had divided after the person's birth.

Sources of cardiac cells

1. Primary cultured
    a. Human – adult from biopsy
    b. Mouse – neonate, adult
    c. Rat – neonate, adult







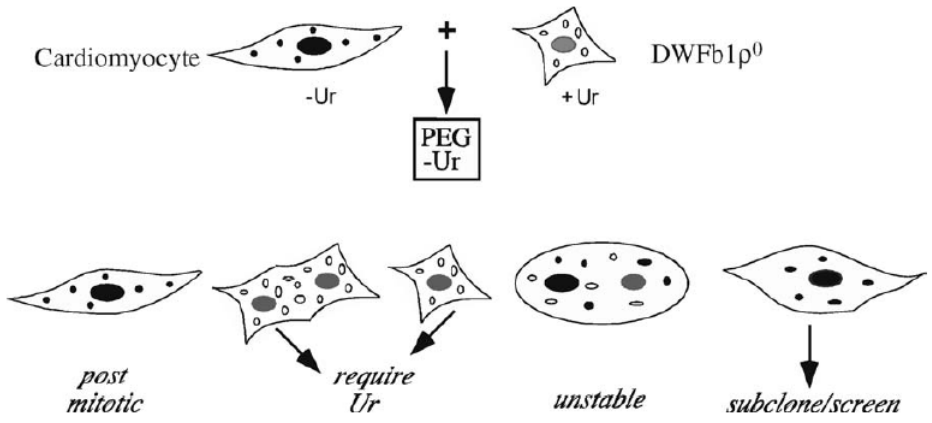
Sources of cardiac cells

2. Immortalized cell line

A. AC16 – human

① Adult human ventricle origin

② Fused with SV40 , having large T antigen



Comparison of AC cells and primary cardiomyocytes in culture

Markers	Primary cardiomyocytes	AC cells
Culture characteristics		
Cell division	NO	YES
Differentiation	NO	YES
Large T-Ag	–	+
Contractile proteins		
α-MHC	+	+
β-MHC	+	+
VMLC-1	+	+
Troponin I	+	+
α-Cardiac actin	+	+
α-Skeletal actin	–	–
Desmin	+	+
Vimentin	–	–
α-Actinin	+	+
Myofilaments	+	+
Sarcomeres	+	–
Membrane proteins		
Desmoplakin	+	+
Intercalated disc	+	–
Gap junctions	+	+
Cx-43	+	+
Cx-40	+ (atrial)	+
Transcription factors		
GATA4	+	+
MYCD	+	+
NFATc4	+	+
Signaling molecule		
BMP2	+	+[a]
Ion channel		
CACNα1C	+	+

[a] After differentiation.

Davidson MM et al., *J Mol Cell Cardiol*. 2005 Jul;39(1):133-47

Sources of cardiac cells

2. Immortalized cell line

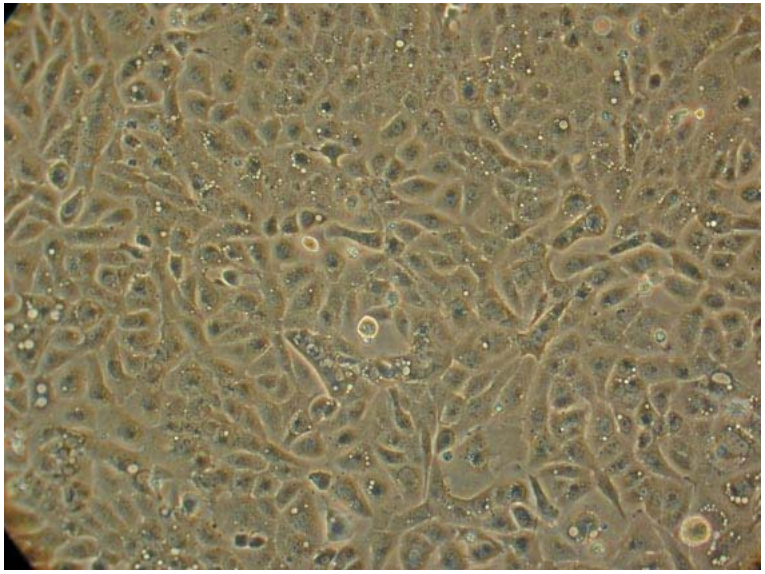
B. HL-1 – mouse

① Retains contractile phenotype

② supplemented with

2 mM L-glutamine

0.1 mM norepinephrine



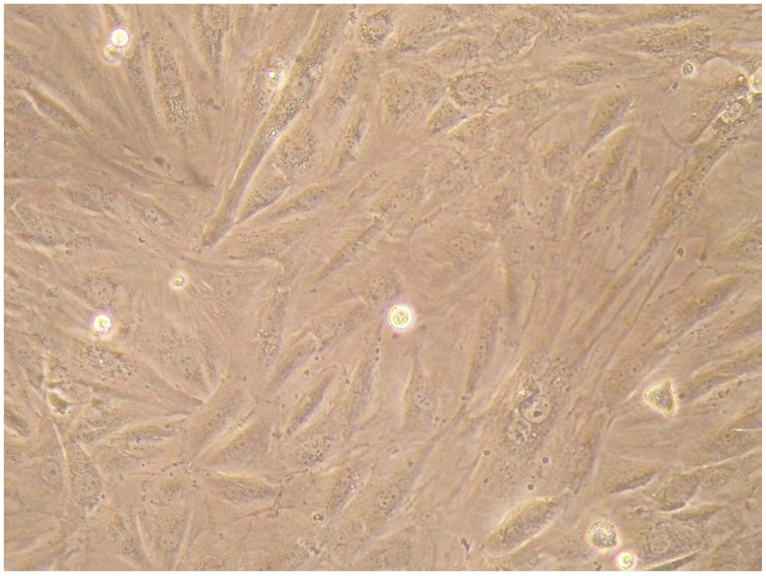
Sources of cardiac cells

2. Immortalized cell line

C. H9c2 – rat

① Avoid to be confluent

② Can be differentiated to skeletal myocyte by serum deprivation or by retinoic acid



Neonatal rat ventricular cardiomyocytes (NRVCMs)

Materials

1. SD Rat pubs (PN 1d ~ 2d)
2. Ads buffer – 120 mM NaCl, 6 mM glucose,

   8 mM NaH_2PO_4, 5 mM KCl,

   0.8 mM $MgSO_4$, 20 mM HEPES, pH7.4
3. Collagenase (type II) – 0.5 mg/ml
4. Pancreatin – 0.6 mg/ml
5. Percoll
6. Gelatin or collagen

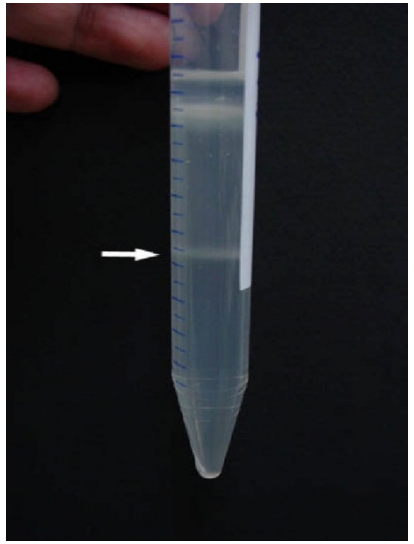
Neonatal rat ventricular cardiomyocytes (NRVCMs)

Methods

1. Isolate heart from rat pubs
2. Remove appendages (use only ventricle)

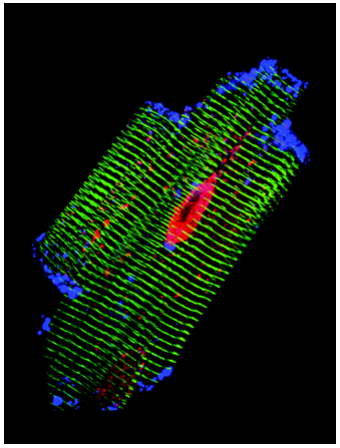
3. Mince the ventricles
4. Enzyme digestion (Collagenase/Pancreatin/Ads buffer)
5. Percoll gradient

   or pre-plating without coating



6. Coat the culture plate with collagen or gelatin
7. Seed the NRVCMs $2x10^6/\phi 60$
8. Check the NRVCMs beating

Adult cardiac myocytes (Mouse)

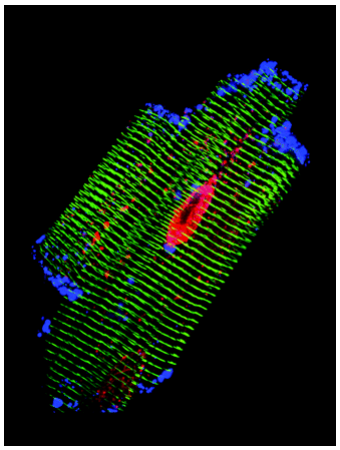


Methods

1. 10X Digestion buffer (DB)

NaCl	130mM
KCl	5mM
Pyruvic acid	3nM
HEPES	25mM
$MgCl_2$	0.5mM
NaH_2PO_4	0.33mM
Dextrose	22mM
Adjust pH to 7.4	

Adult cardiac myocytes (Mouse)



Methods

2. Digestion Buffer A

    50mL – 1x DB with 75ul – EGTA (100mM EGTA)

3. Digestion Buffer B

    25mL – 1x DB with 1.25uL – $CaCl_2$ (1M)

    1mg – Protease, 25mg – Collagenase

4. Collection buffer (37^{o}C)

    2.5mL – 1X DB with 1.25ul – $CaCl_2$ (1M)

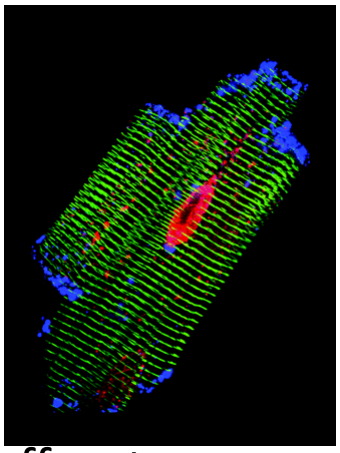
    0.5mg – Protease, 15mg – Collagenase, 125mg – BSA

5. Neutralization Wash Buffer

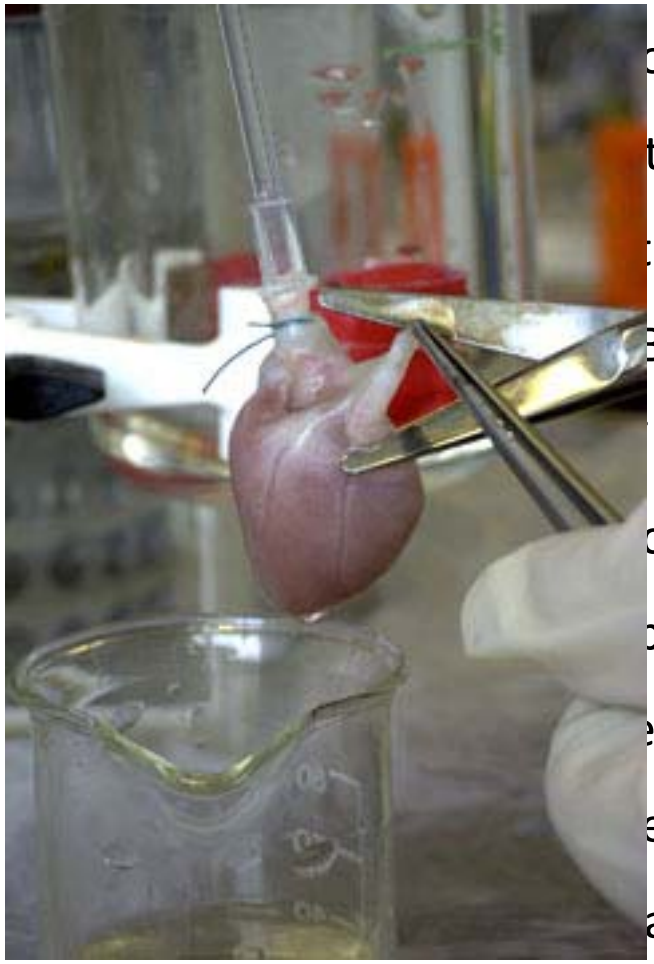
    25mL – 1X DB with 6.25ul – $CaCl_2$ (1M)

    250mg BSA

Adult cardiac myocytes (Mouse)



Methods



ouse and keep cold with Buffer A

th ice cold Buffer A

h 37°C fluid (Buffer A) – Langendorff system

3 from A after blood is washed from the heart

with scissors and place into Collection Buffer(CB)

cle into small pieces while placing into the CB

ieces sink to the bottom

supernatant

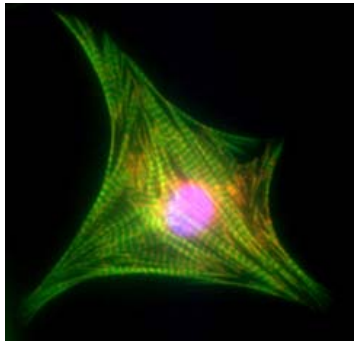
et from the supernatant for 15 minutes at RT

atant and wash pellet by adding the

Neutralization buffer.

Flynn JM et al., *J Vis Exp. 2011 Dec 28;(58):e3302. doi: 10.3791/3302.*

Limitations of NRVCMs or H9c2



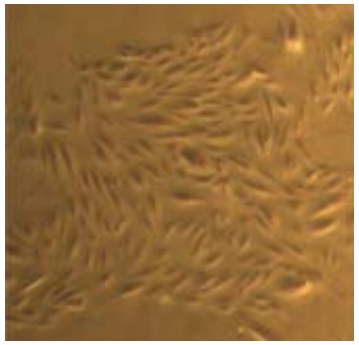
1. NRVCMs

    a. Risk of bacterial contamination

    b. Yield

    c. Subculture

    d. Extremely low transfection efficiency – viral delivery system



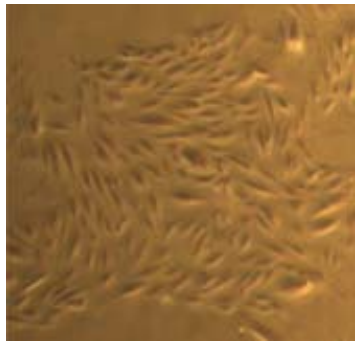
2. H9c2

    a. Myoblast cell

    b. Lack of cardiac phenotype

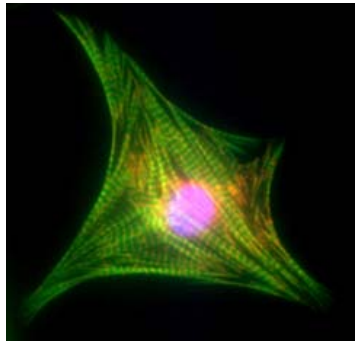
Usage of NRVCMs or H9c2

1. H9c2



a. General cell works except chemical treatment

2. NRVCMs



a. ICC

b. Cell size measurement

c. Promoter assay

d. cell works, especially chemical study

- when using viral delivery system, NRVCMs is the cell of choice.

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- Cell Lines (309)

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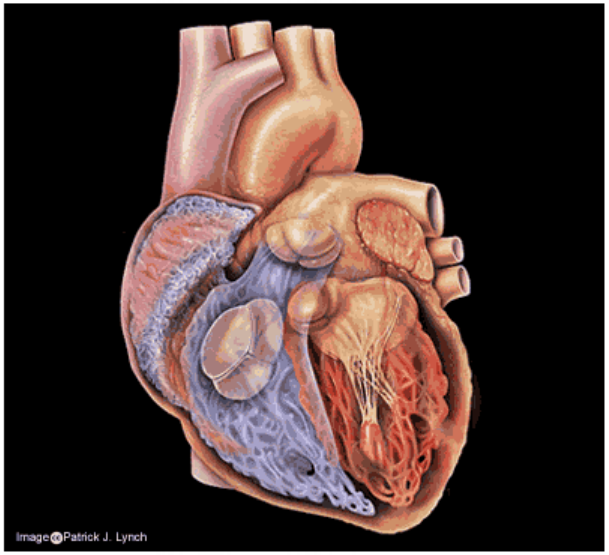
Cardiac Cells for the Advancement of Heart Research

The cardiac system provides the mechanical means for delivering nutrients to the tissues and organs of the body. Study of heart cells is key to identifying pathologies that injure or kill millions of people every year. Cell Applications, Inc. provides cardiac cells of various lineages for the advancement of heart research.

Examples of Research with Cell Applications, Inc.'s Cardiac Cells:

- **Human Aortic Endothelial Cells (HAOEC)** have been used as an *in vitro* model for the study of morphological and ultra-structural changes in the development of atherosclerosis.
- **Human Aortic Smooth Muscle Cells (HAOSMC)** are capable of synthesizing collagen, elastin, myosin and glycosaminoglycan.
- **Human Cardiac Fibroblasts (HCF)** has been used to study the effects of TNF-alpha and angiotensin (Ang) II levels on fibroblast function.
- **Human Coronary Artery Endothelial Cells (HCAEC)** are crucially involved in the regulation of coronary blood

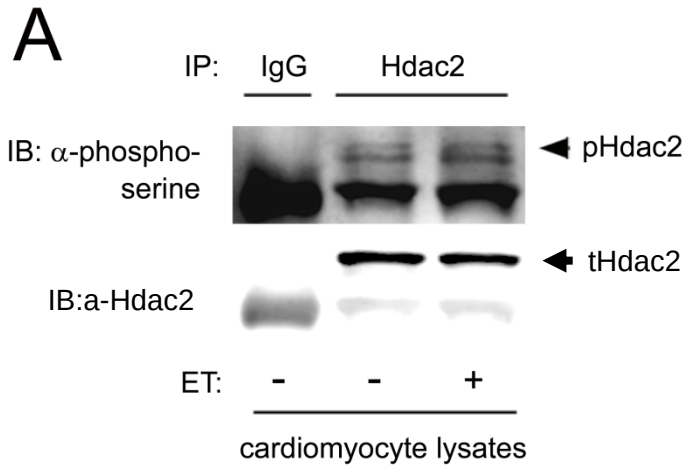


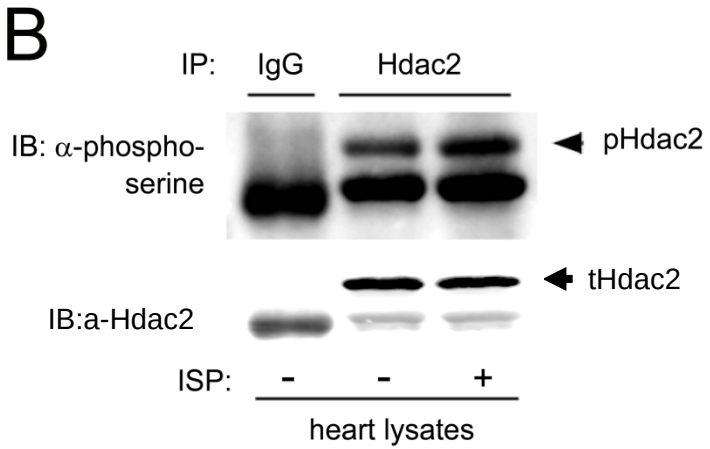
http://www ɔTrader, Inc)

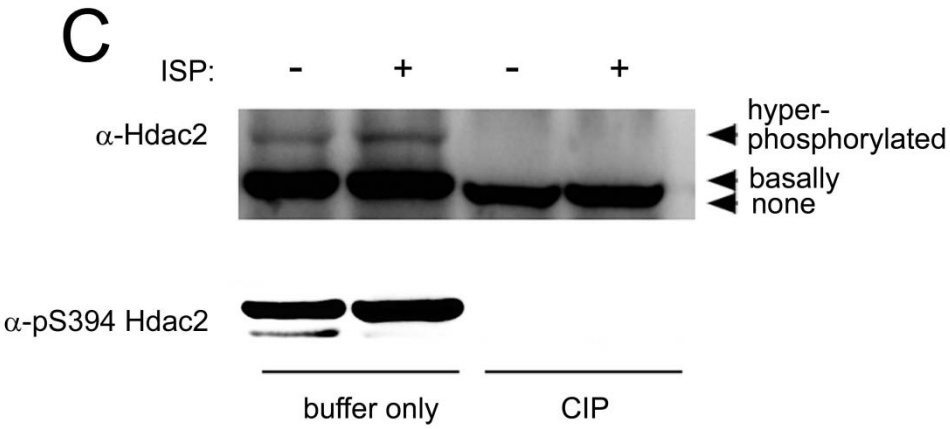
http://www.cellapplications.com/primary-heart-cells (**Abba BioScience**)

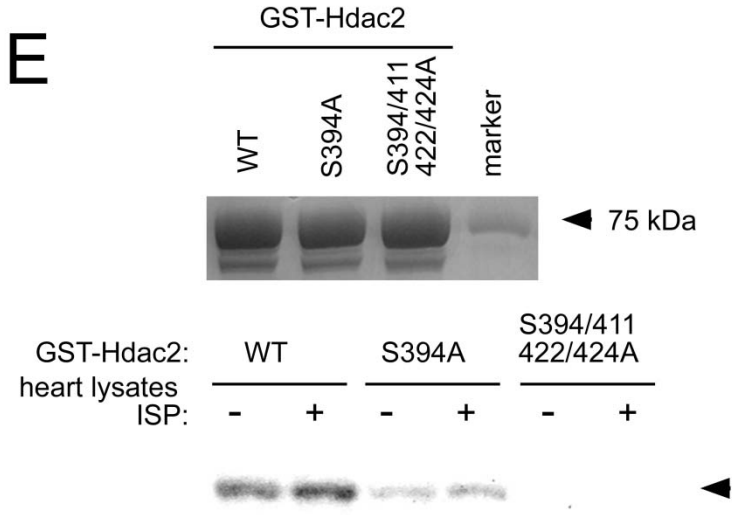
Examples

Hypertrophic stimuli phosphorylate Hdac2 S394

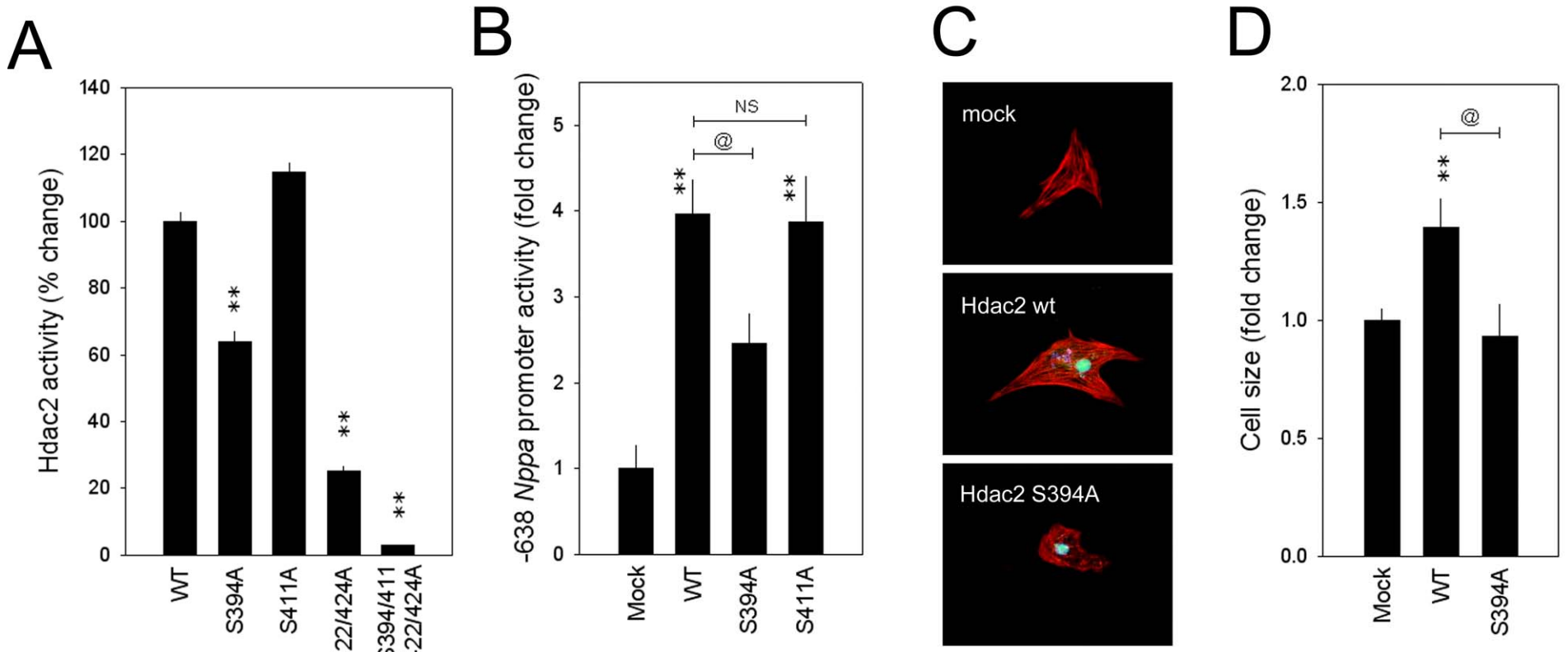




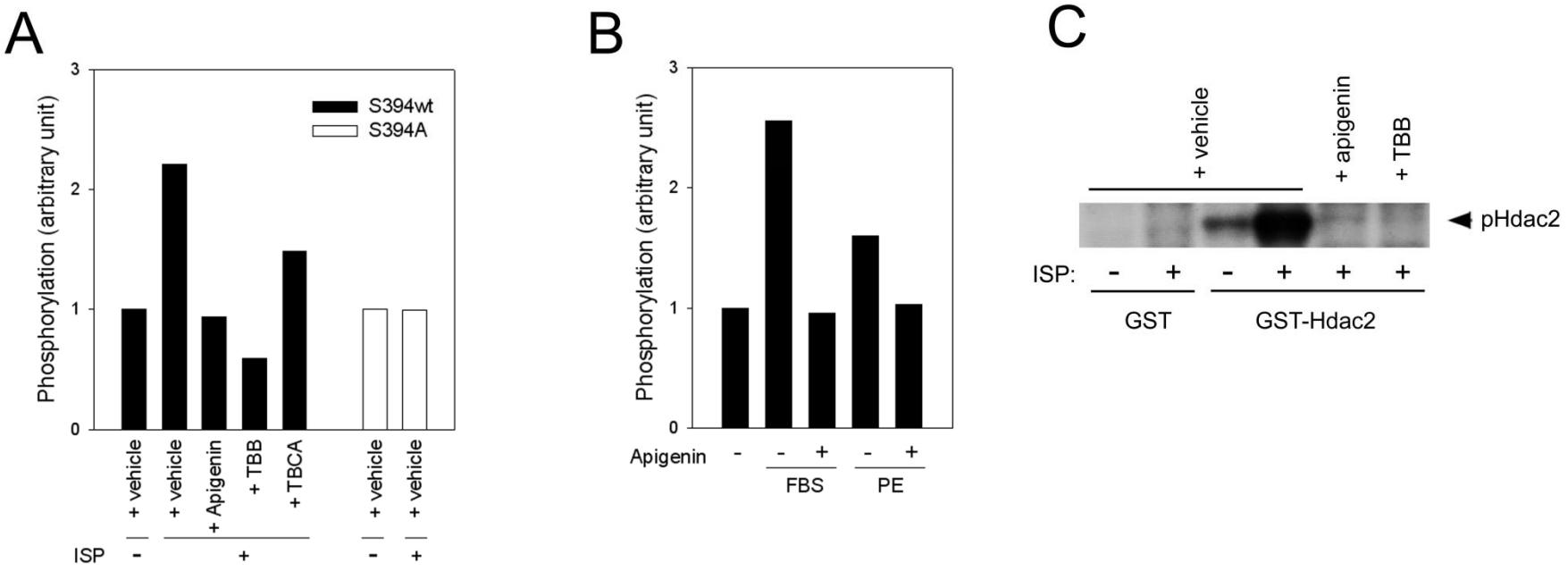




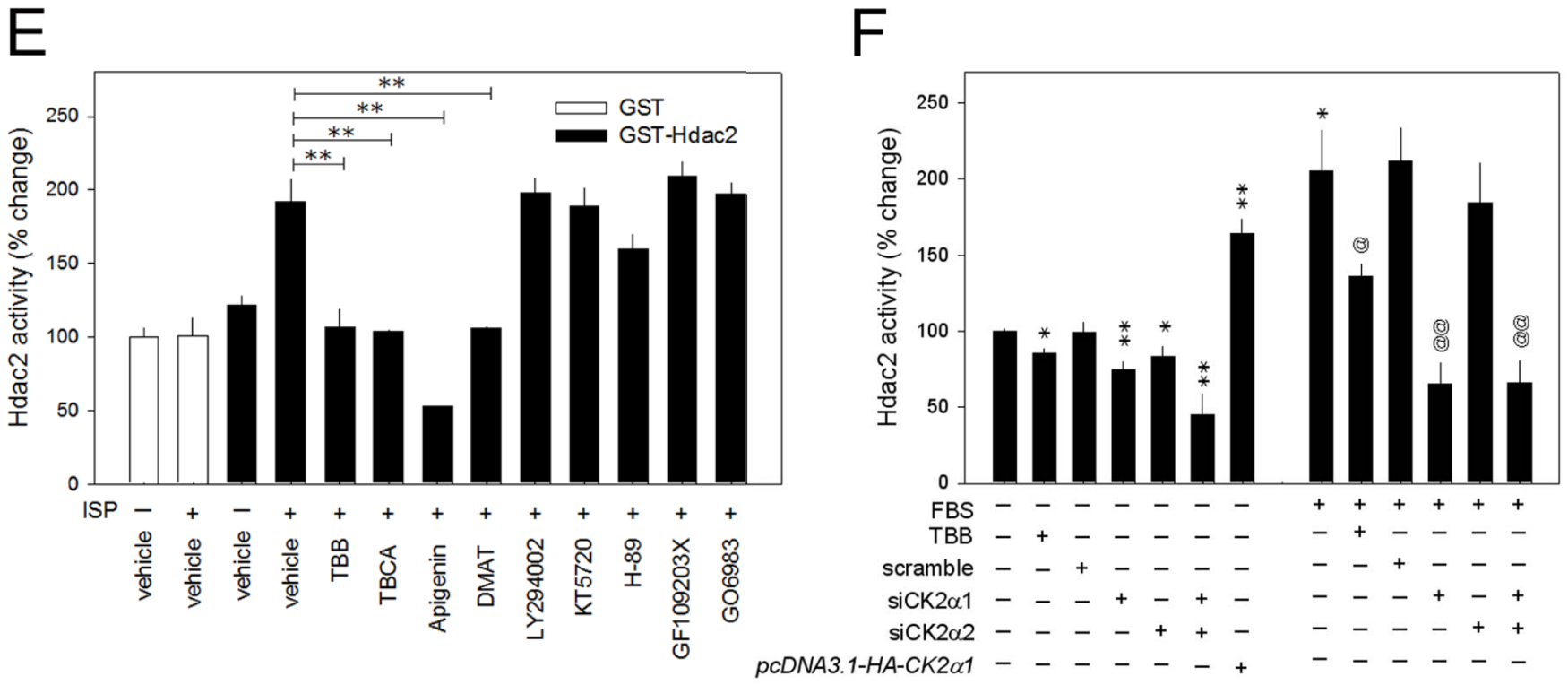
Phosphorylation of HDAC2 S394 is required for the development of cardiac hypertrophy



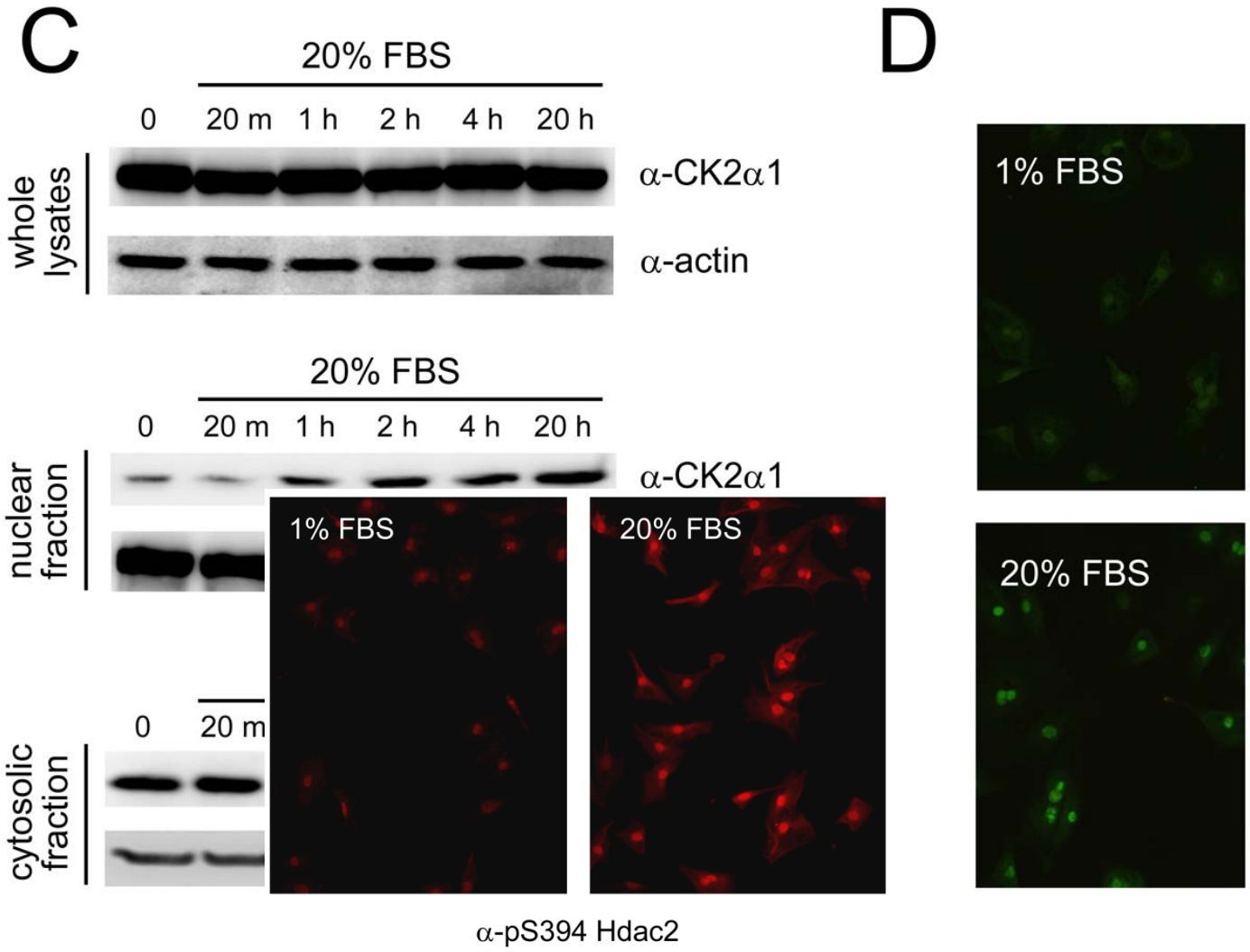
Casein Kinase 2 phosphorylates Hdac2 S394



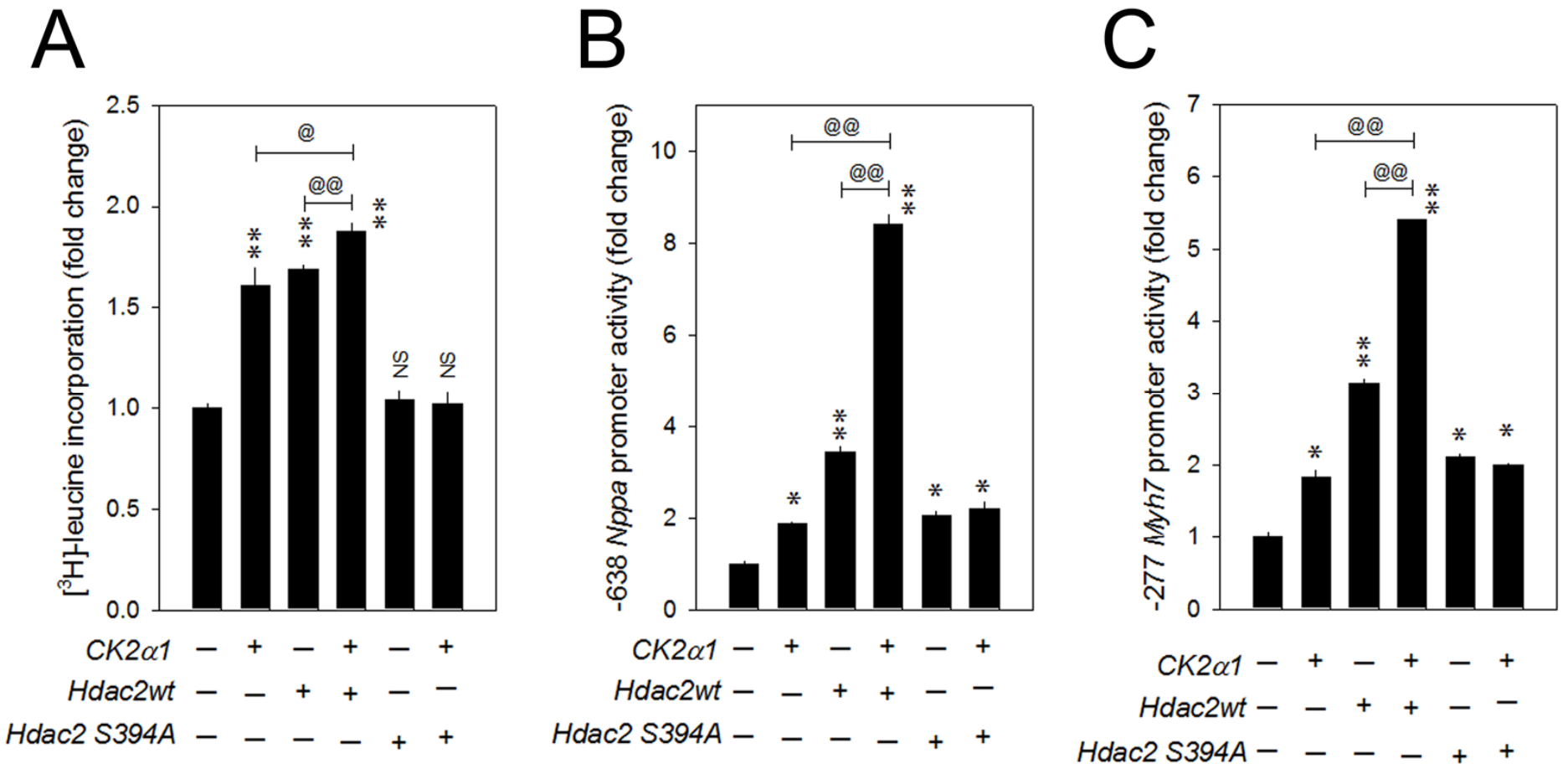
Casein Kinase 2 phosphorylates Hdac2 S394



CK2 is activated in response to hypertrophic stimuli



Hdac2 S394A blunts the hypertrophic phenotypes of *CK2α1*



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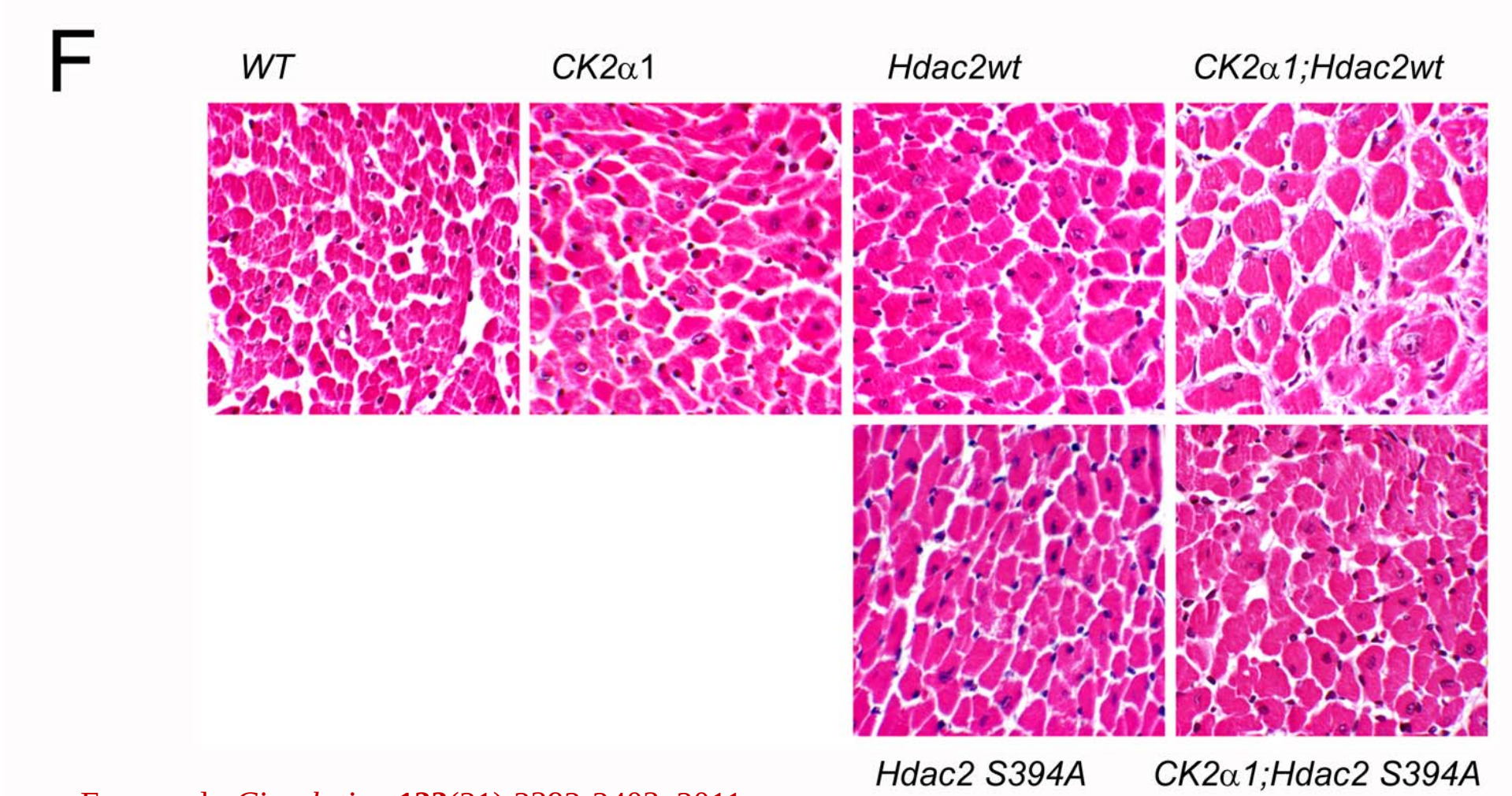
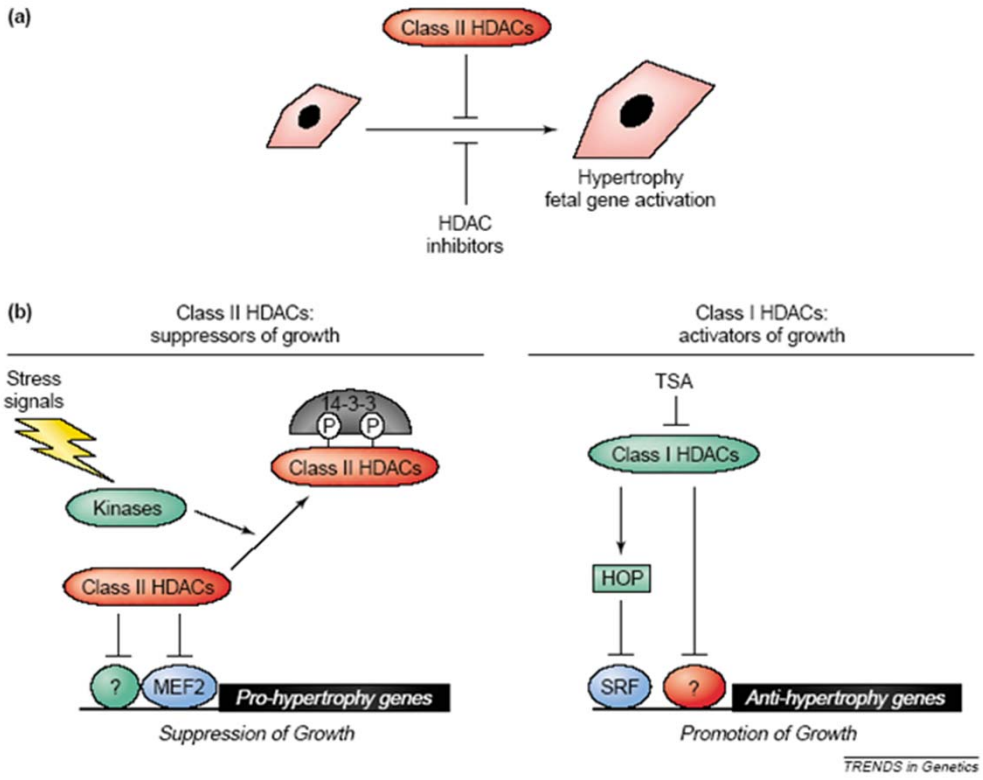
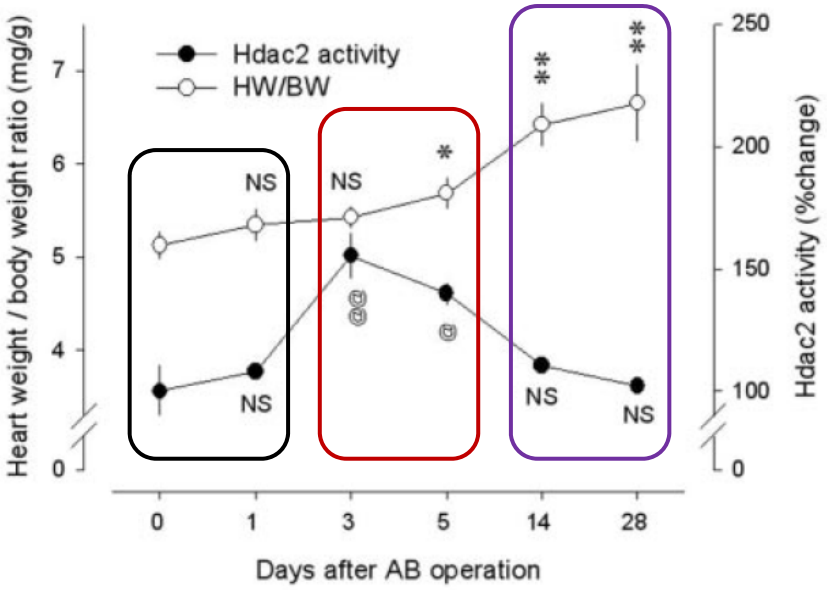


Eom et al., *Circulation* **123**(21):2392-2403, 2011

Crosstalk between Hdac2 and Class IIa HDACs

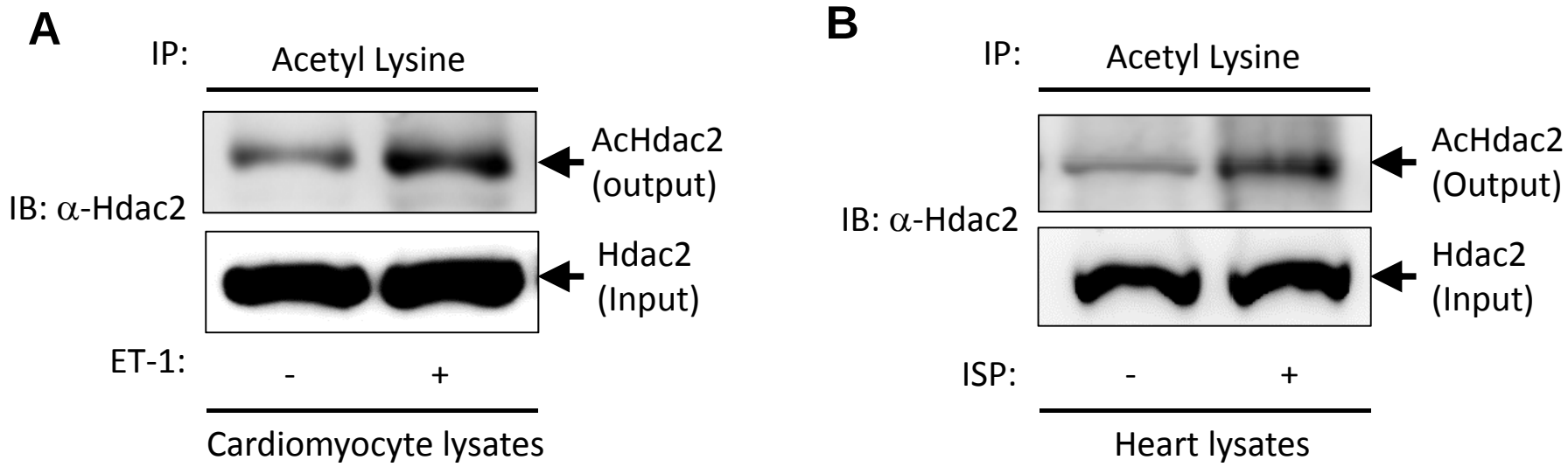


McKinsey TA and Olson EN. *Trends Genet*, **20**(4):206-13, 2004

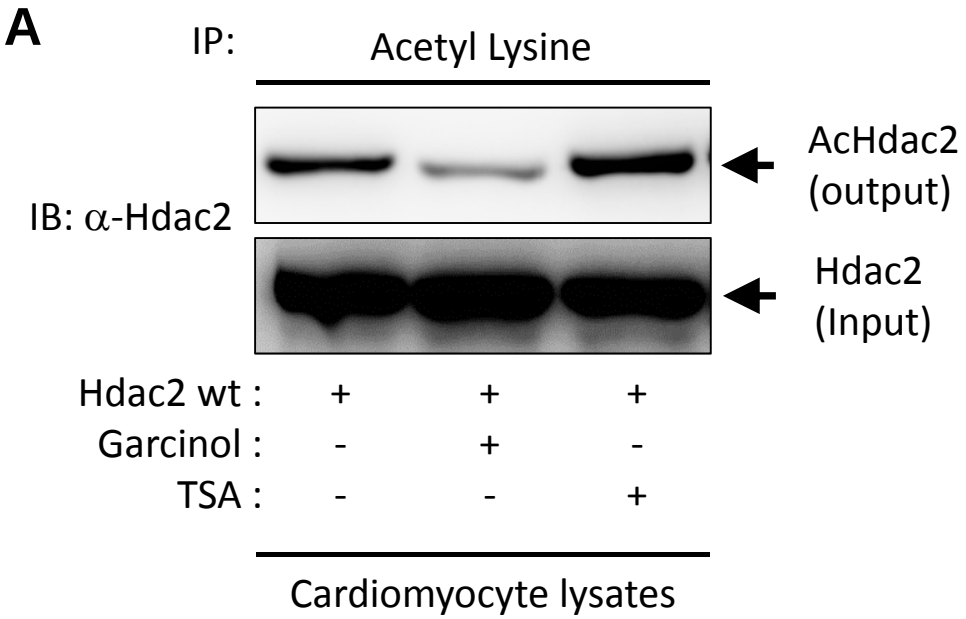


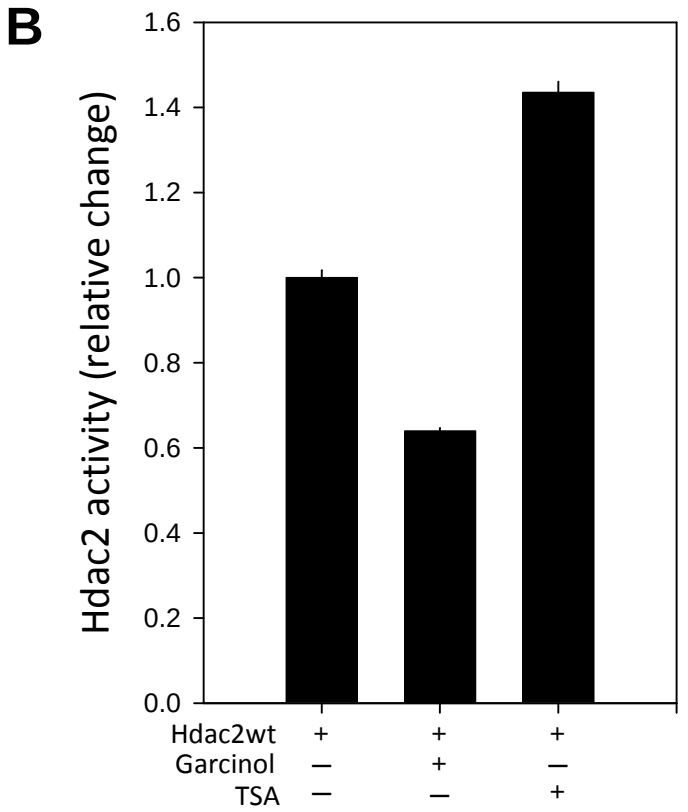
Kee and Eom et al., *Circ Res*, **103**(11):1259-69, 2008

Hdac2 is acetylated in the development of cardiac hypertrophy



Hdac2 acetylation is reduced by Garcinol but increased by TSA

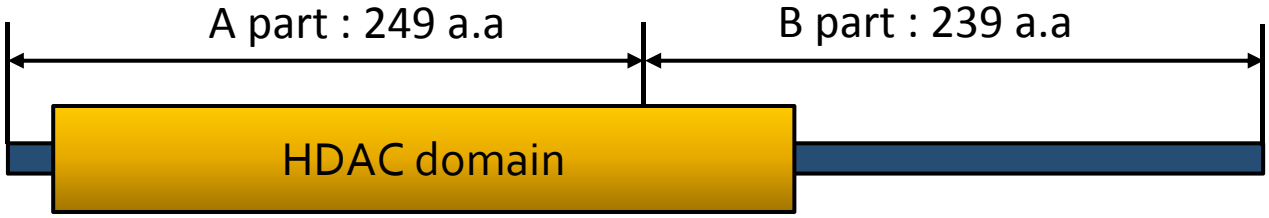


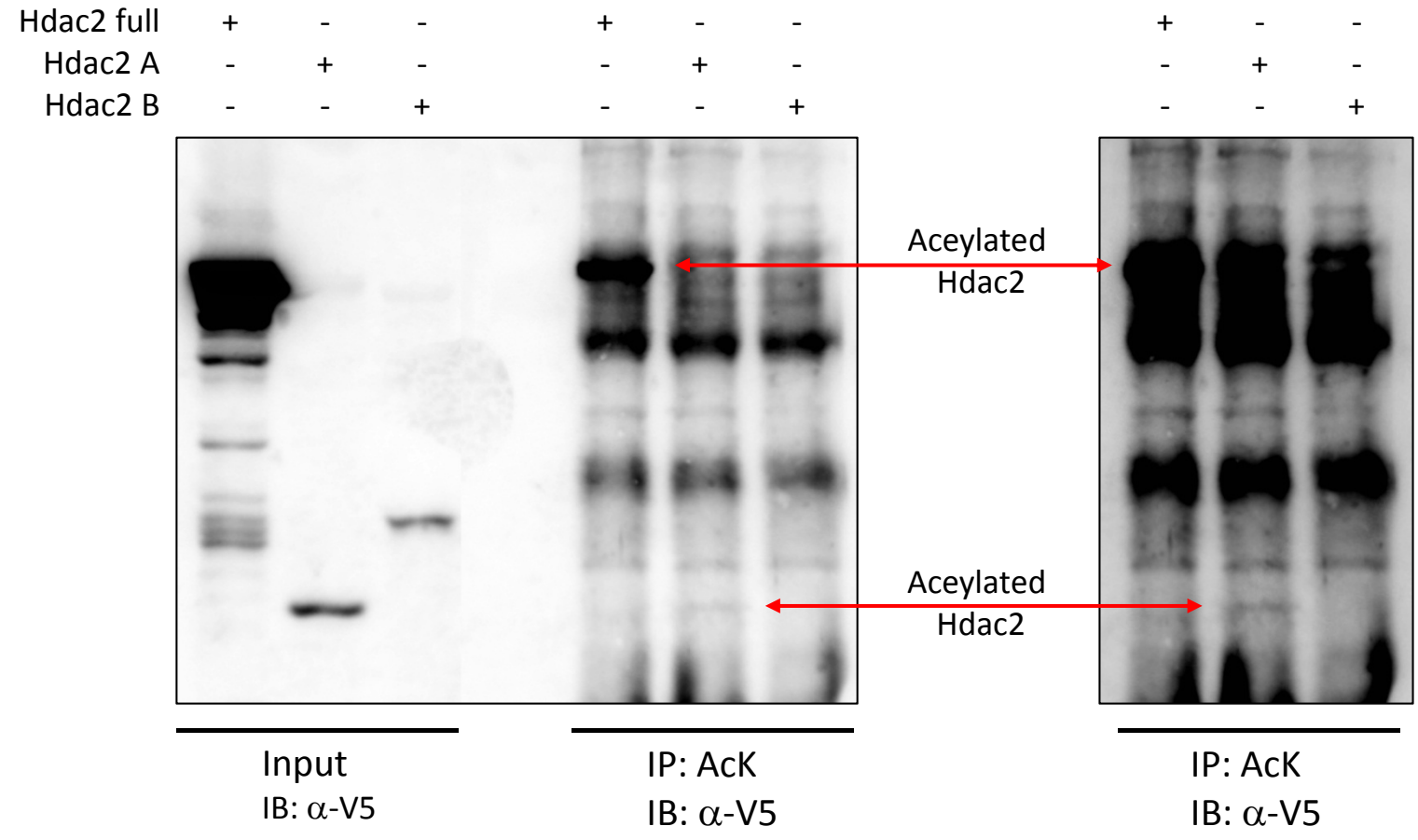


Garcinol : HAT(histone acetyl transferase) inhibitor
TSA : HDAC(histone deacetylase) inhibitor

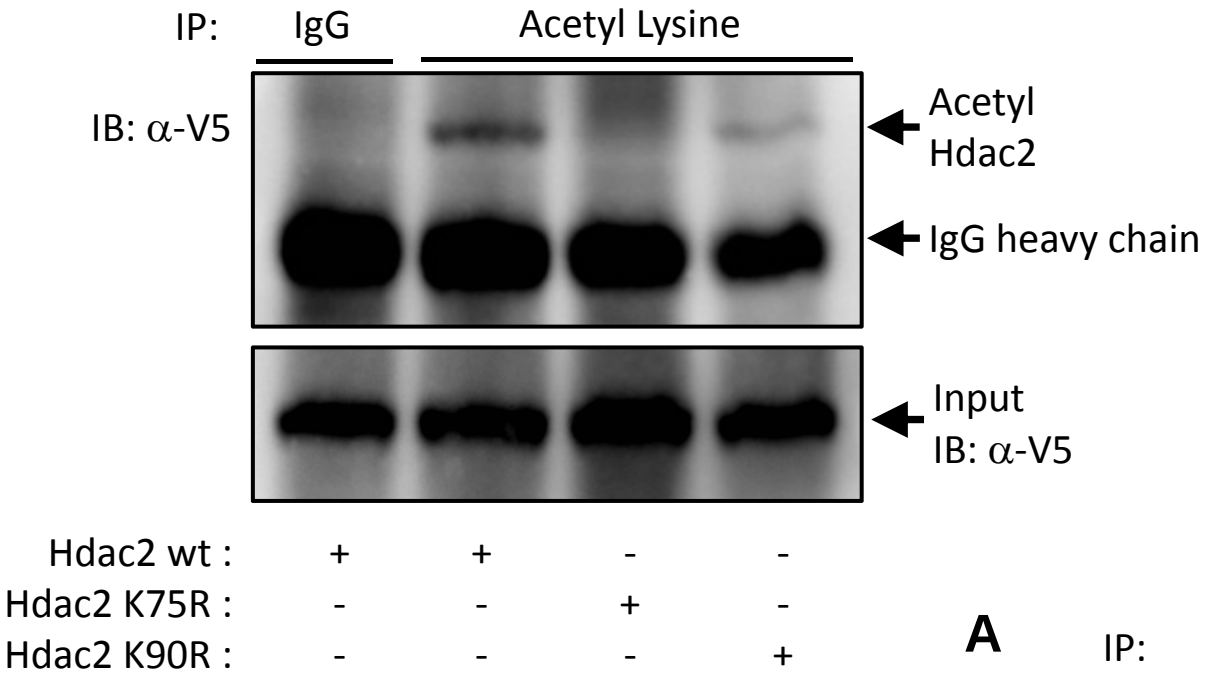
Which residue(s) is(are) the acetylation target(s)?

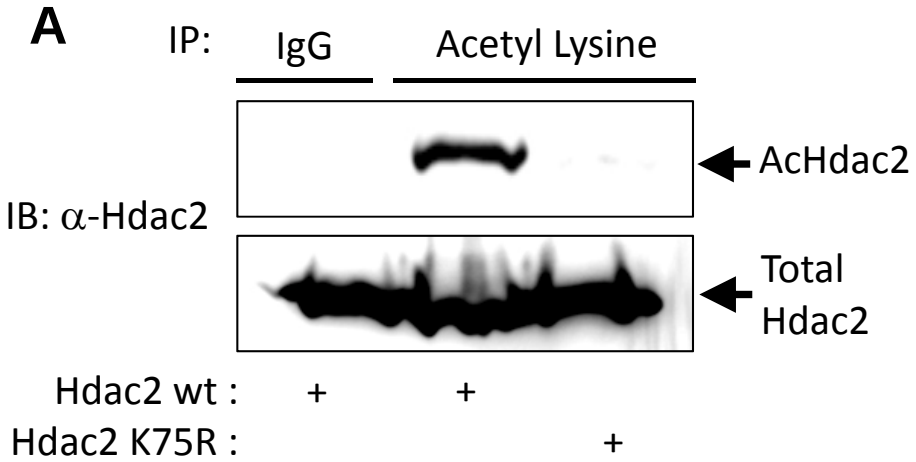
Acetylation target of Hdac2 is located on the A part





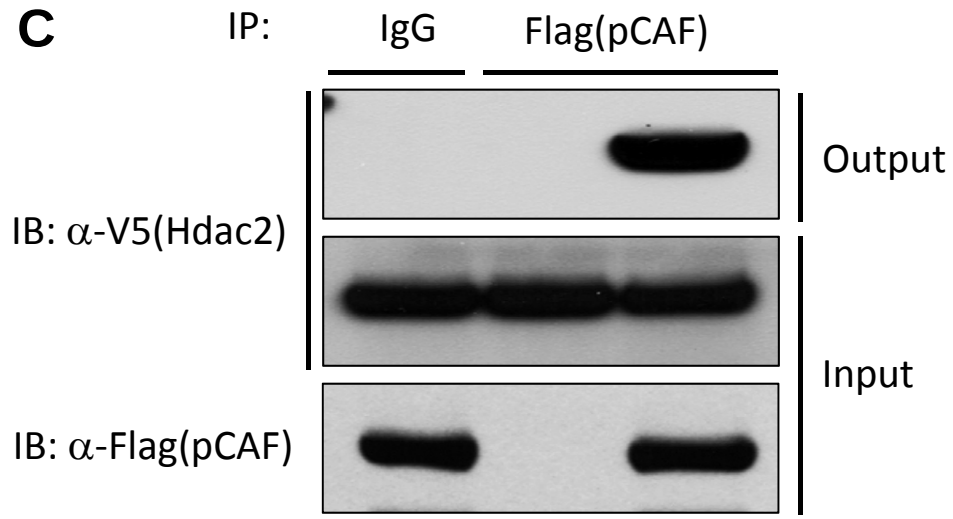
K75 is the target of acetylation

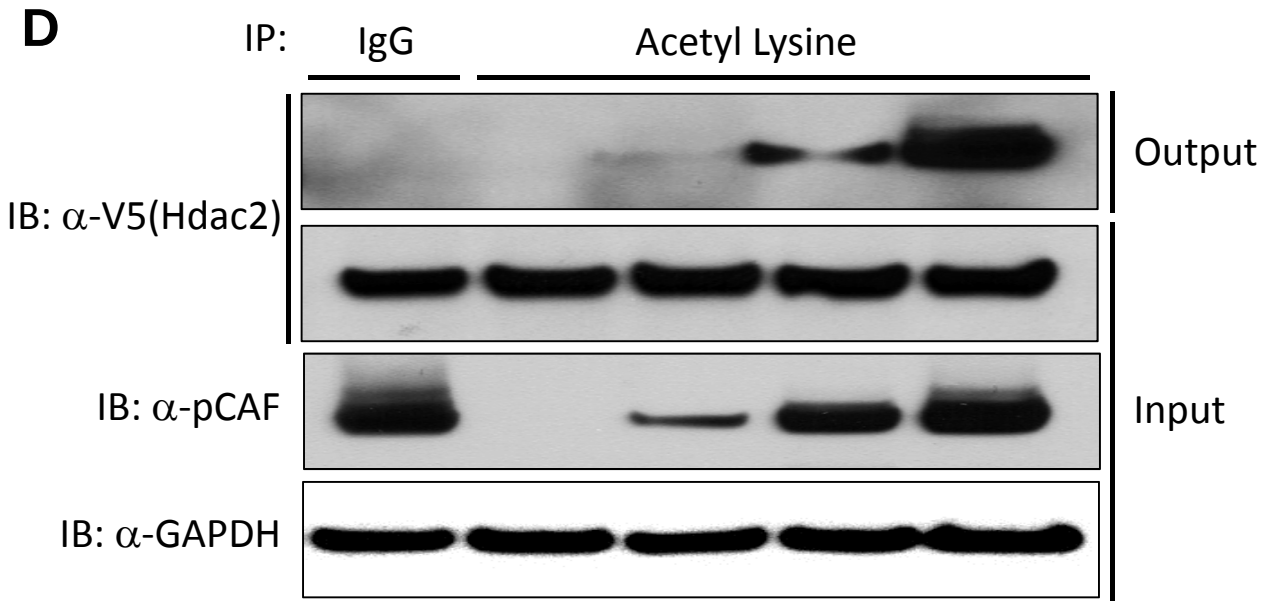




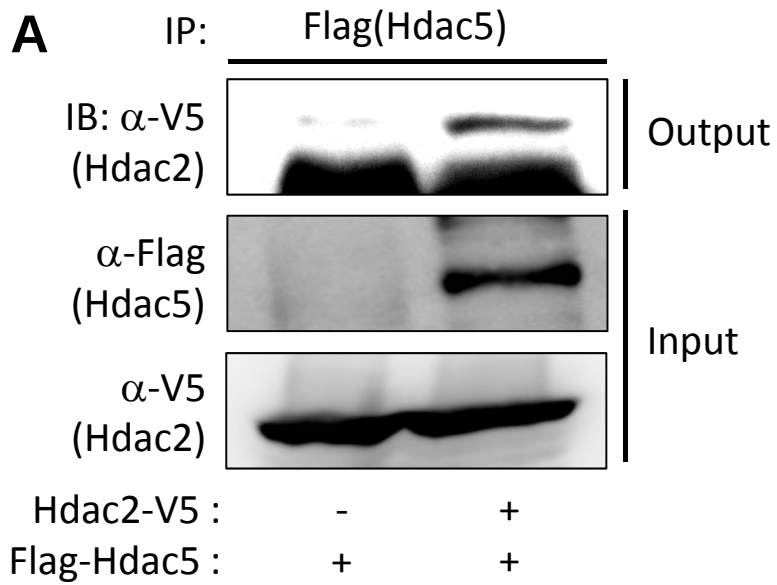
Which HATase?/Which HDAC?

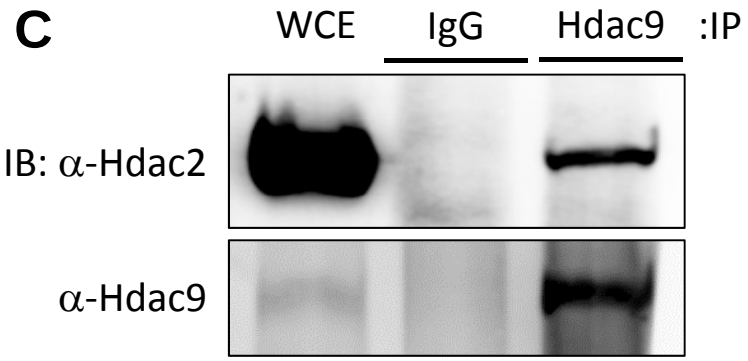
P300/CBP-associated factor binds to Hdac2 for actetylation

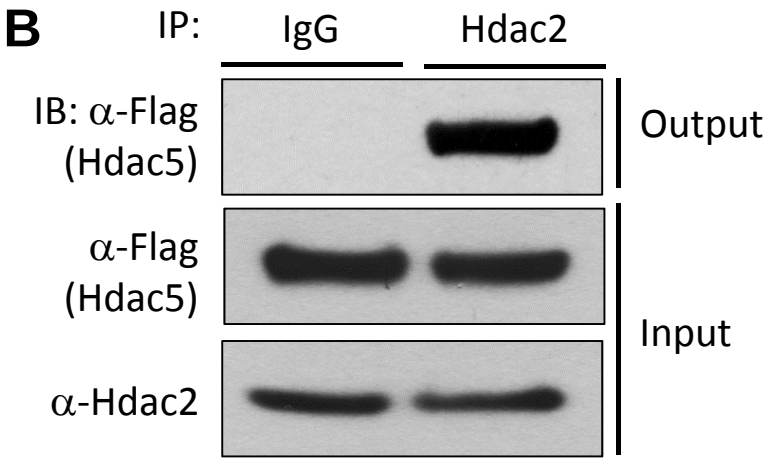


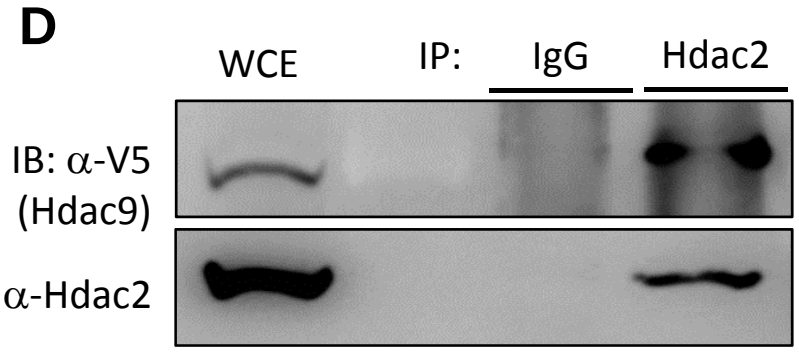


Class IIa HDAC bind to Hdac2

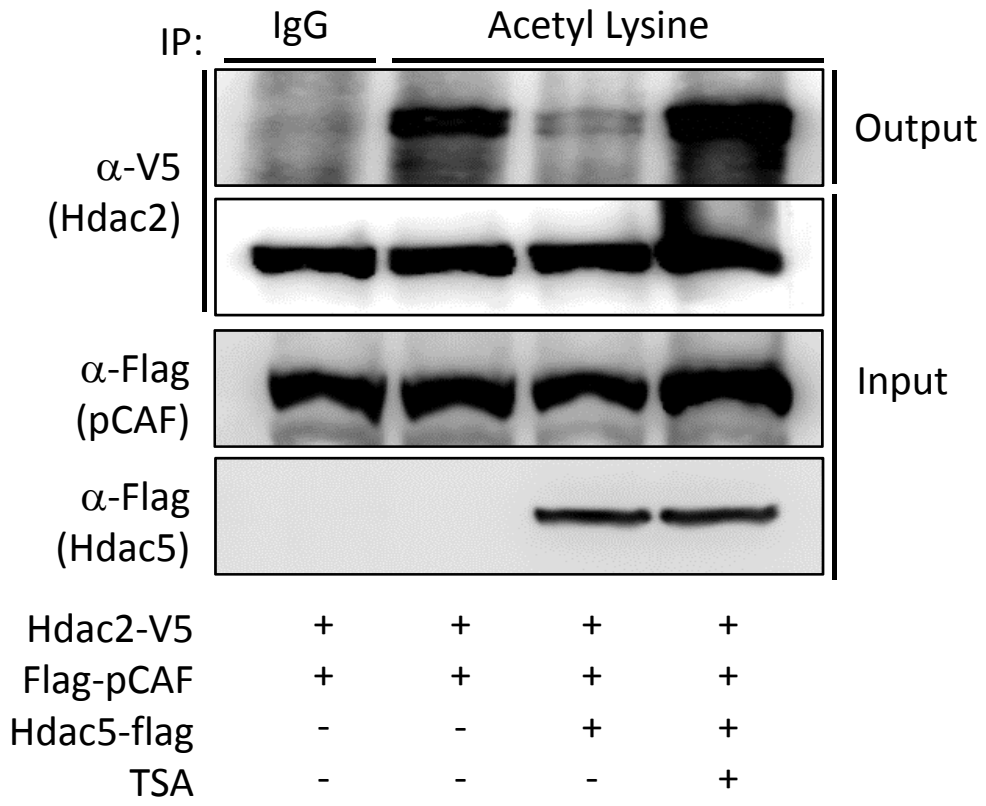






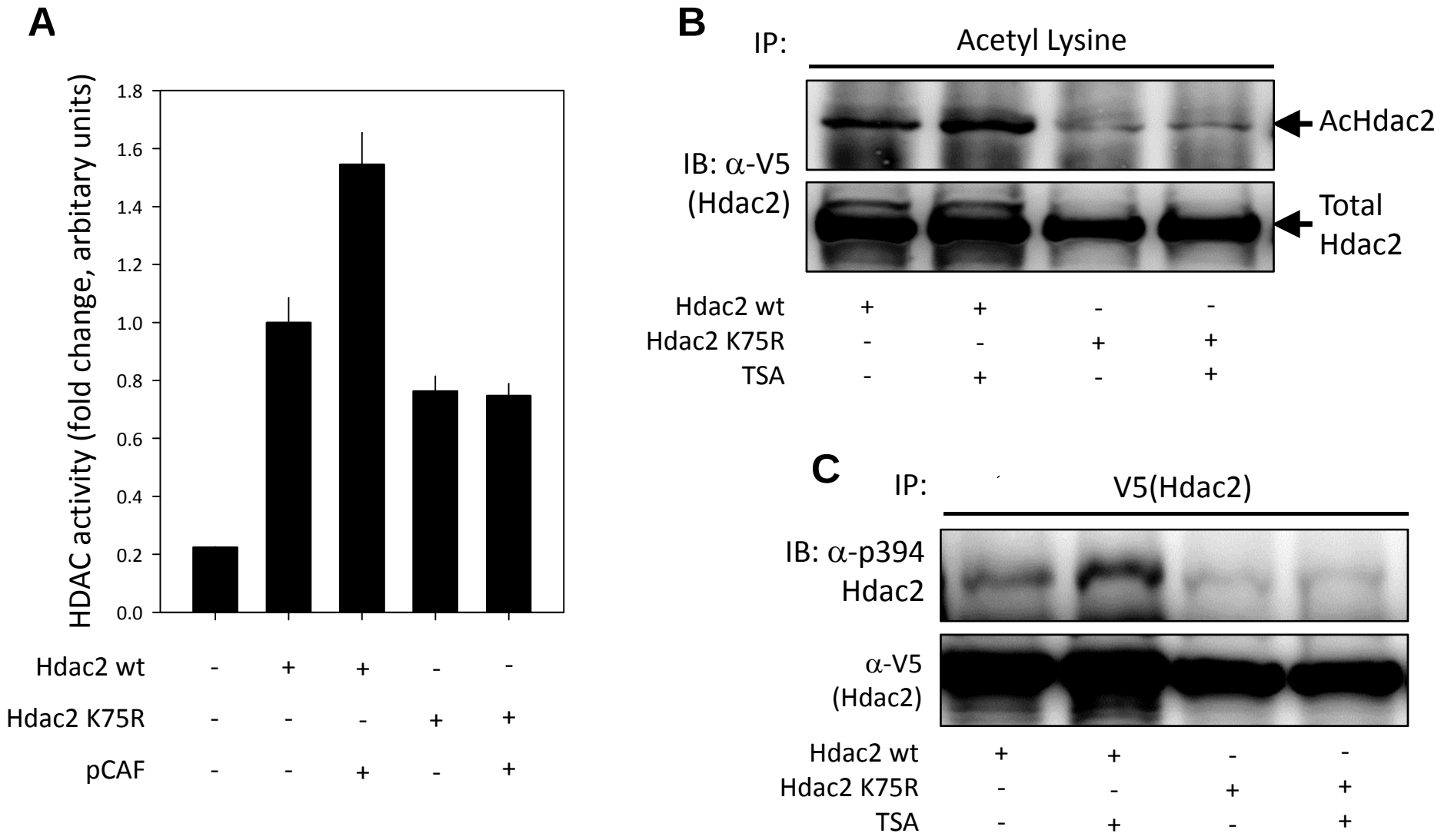


Hdac5 deacetylase Hdac2, which is completely reversed by nonspecific HDAC inhibitor

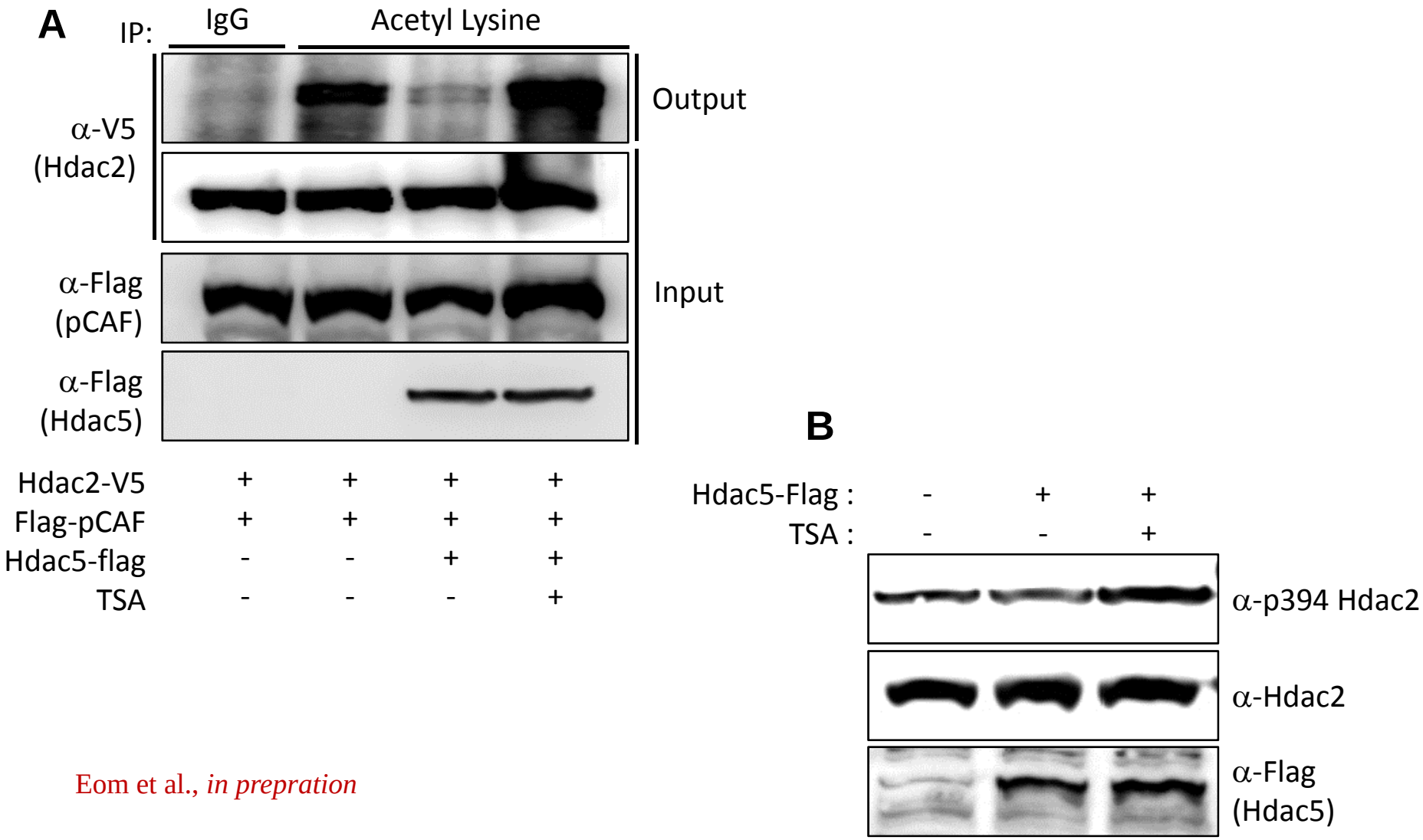


How Acetylation affect to enzymatic activity of Hdac2??

Acetylation of Hdac2 affects phosphorylation status

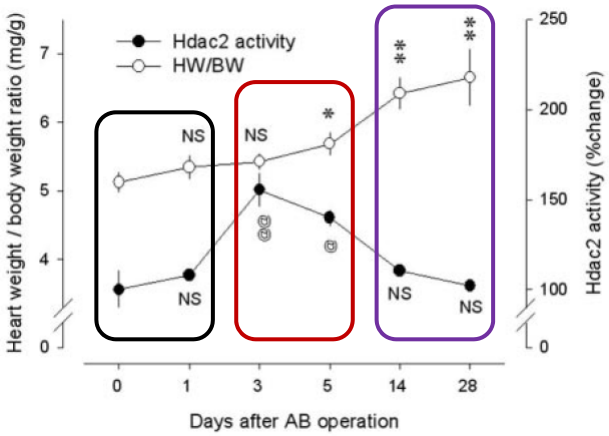


Acetylation of Hdac2 affects phosphorylation status

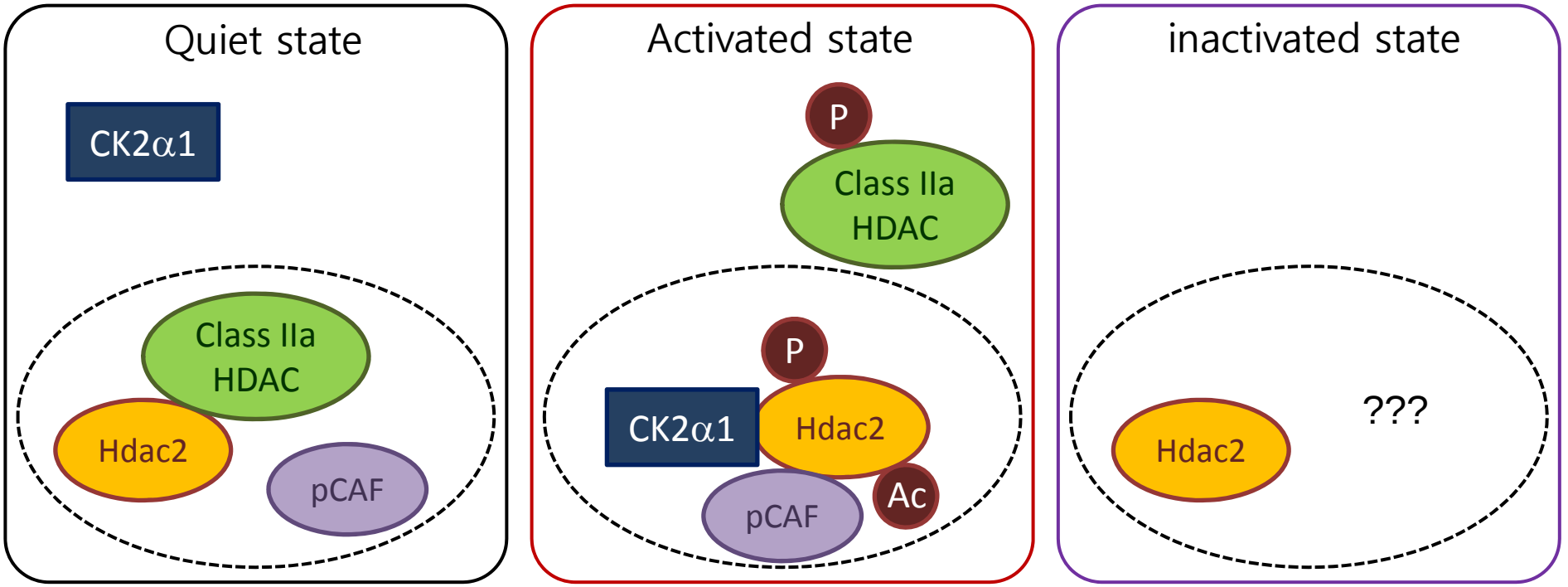


Eom et al., *in prepration*

Working hypothesis



Kee and Eom et al., *Circ Res*, **103**(11):1259-69, 2008



Thank you for your attention!!