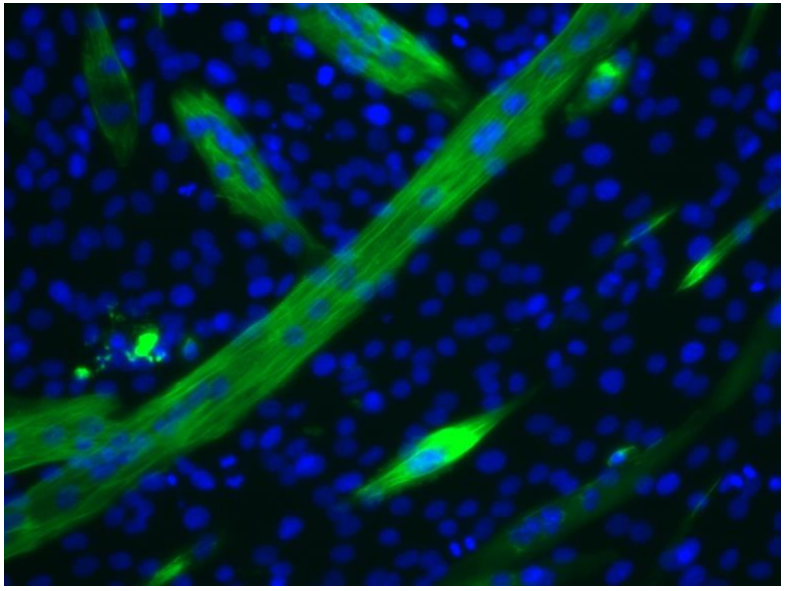
Enhancement of C2C12 myoblast proliferation and differentiation by diarylheptanoid form *Curcuma comosa* Roxb.



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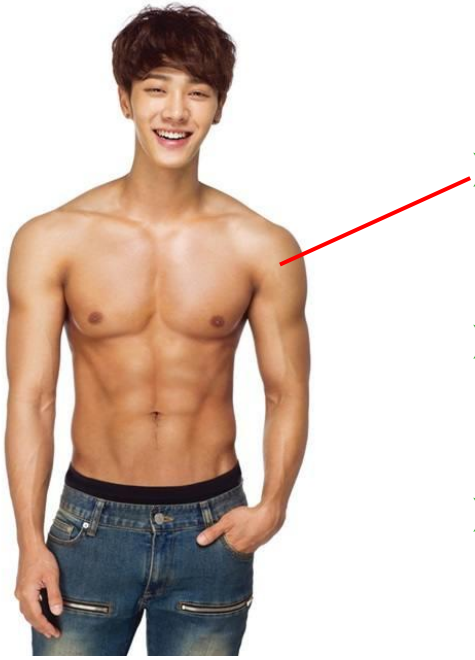


Muscle regeneration

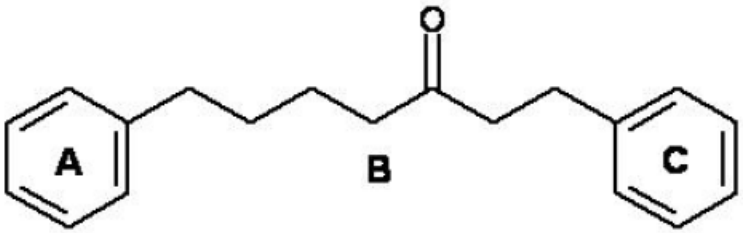
- Skeletal muscle plays an important role in normal daily activities.
- Loss of function of skeletal muscle would, therefore, affect quality of life. --- self-repair after injury---
- Muscle regeneration --- role of satellite cells---

Pavlath *et al*, 1999	Systemic administration of curcumin stimulates muscle regeneration after injury
Tiidus PM *et al*, 2003	Estrogen can protect muscle damage and inflammation after strenuous exercise
Enns DL *et al*, 2008	Estrogen activates satellite cells via estrogen receptors to proliferate in rat skeletal muscle following downhill running

The interest of using phytoestrogens as alternatives to the steroid hormone is dramatically increased. ----less adverse effects----

Diarylheptanoid (DHN)

http://www.bloggang.com/mainblog.php?id=sasiseesom&month=05-07-2012&group=16&gblog=50



- **2 aromatic rings tethered by 7 carbon chain and having various substituents.**

- **Suksamran *et al.* (2008): exhibited estrogenic activity comparable to phytoestrogen genistein**

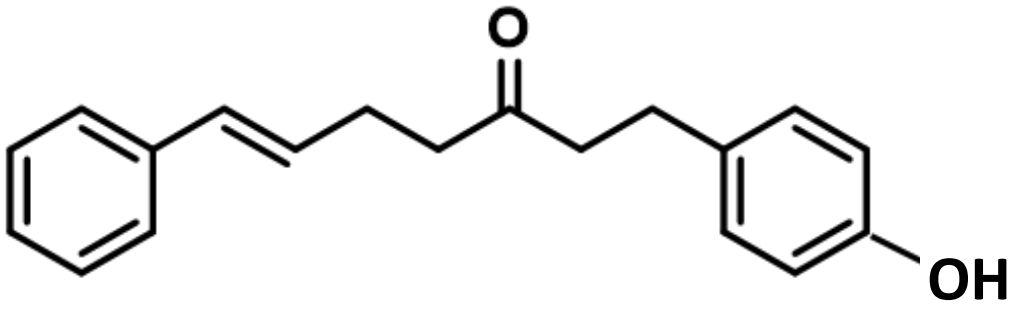
- **exhibit numerous biological activities and have been used as traditional medicine in Asian countries.**

✓ Anti-apoptosis	Zhi *et al*. 2012
✓ Hepatoprotection	Kim *et al*. 2004
✓ Anti-bacteria	Ding *et al*. 2010
✓ Anti-osteoporosis	Arthit *et al*. 2012
✓ Melanogenesis inh.	Watana *et al*. 2012
✓ Anti-cancer	Pawinee *et al*. 2011
✓ Anti-inflammation	Mayer *et al*. 2012

we hypothesize that diarylheptanoids enhance myoblast proliferation and/or differentiation.

Objective

To test the effects of diarylheptanoid on myoblast proliferation and differentiation, and investigate its mechanism.

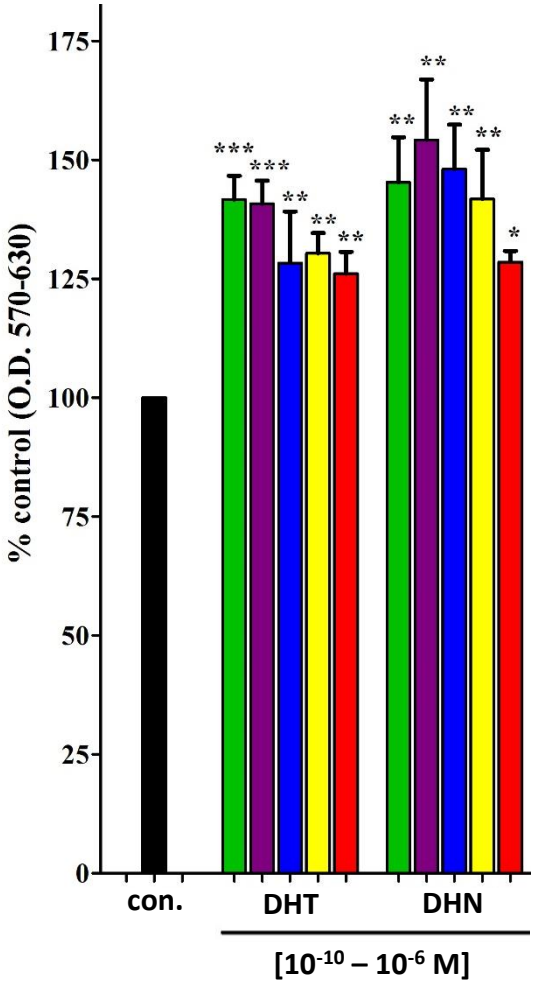


1-(4-hydroxyphenyl)-7-phenyl-(6E)-6-hepten-3-one

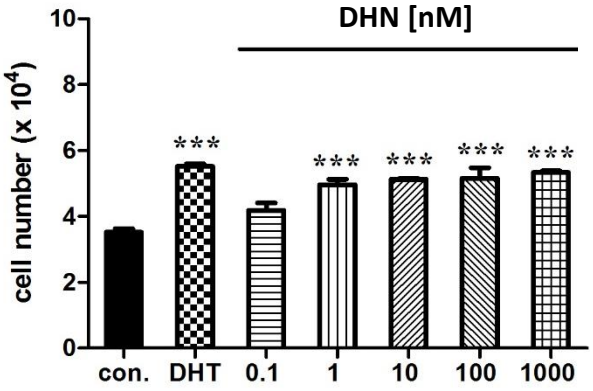
- **Natural and pure compounds**

Proliferation : DHN enhances myoblast proliferation

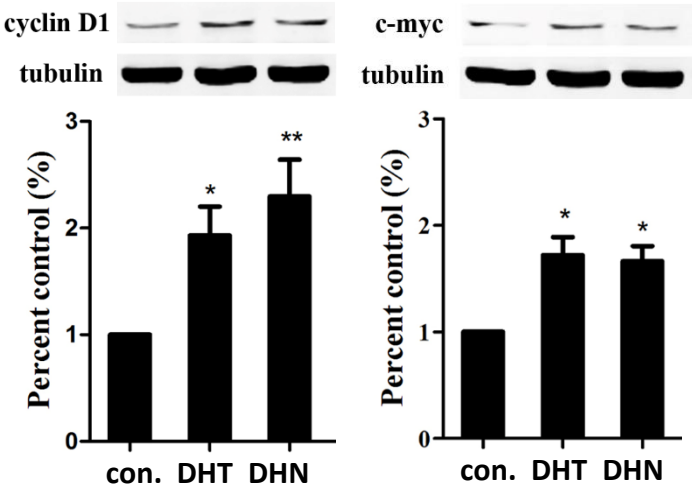
MTT assay



Cell counting

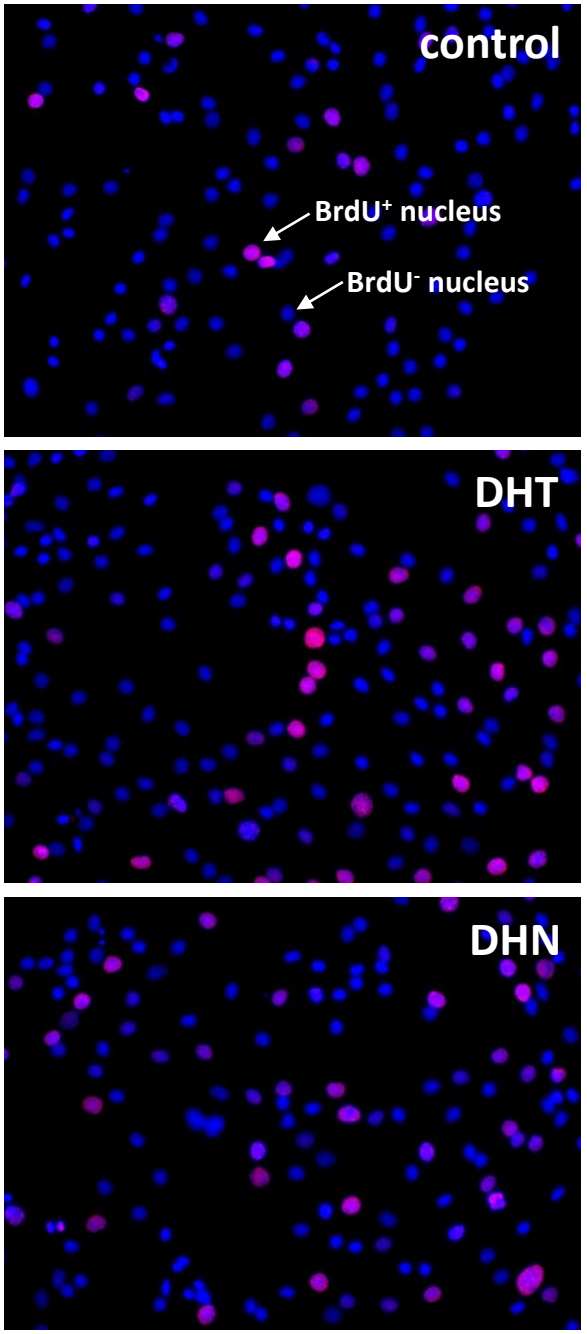


Protein expression

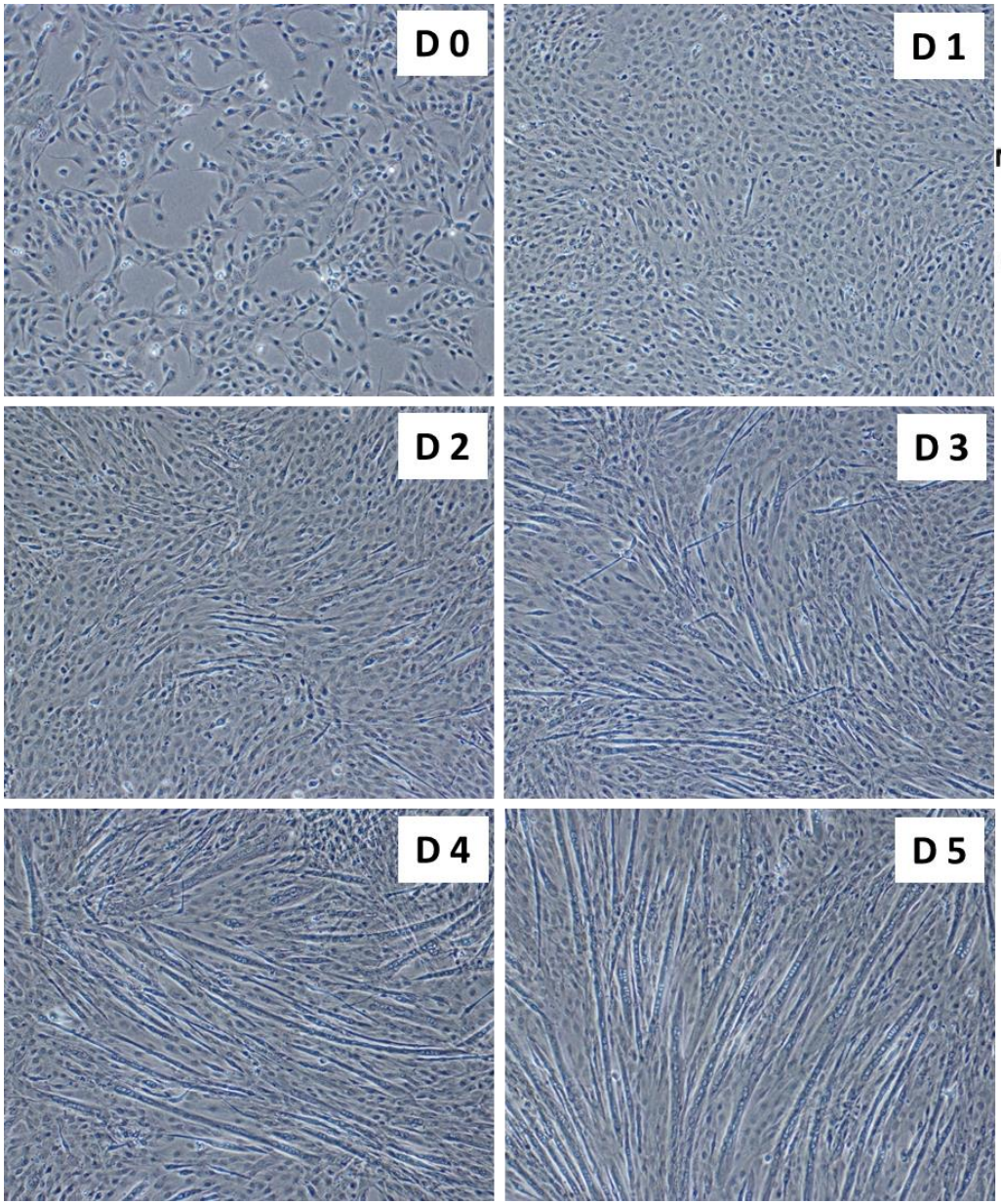


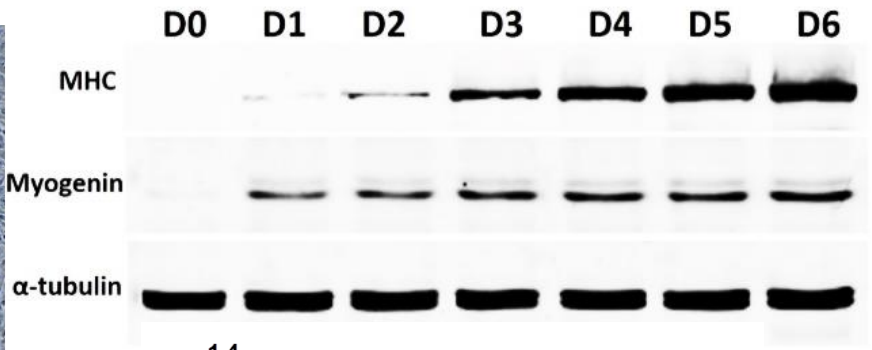
(cyclin D1 and c-myc; markers for cell division)

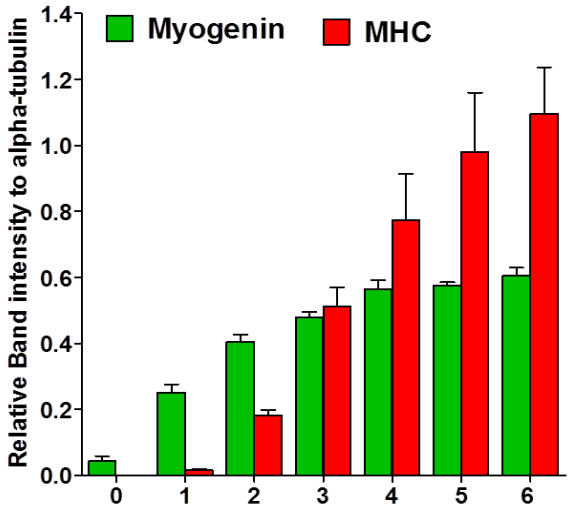
BrdU incorporation assay



Differentiation : Progression of myoblast differentiation



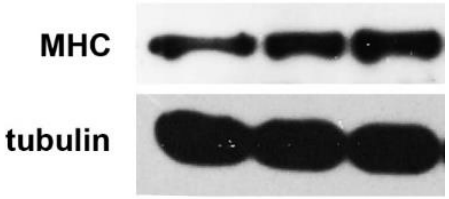


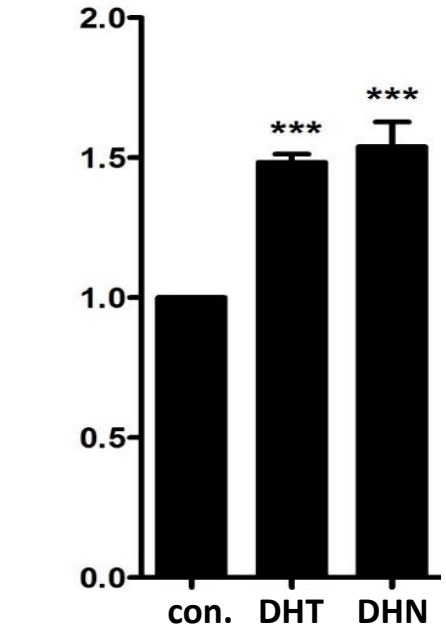


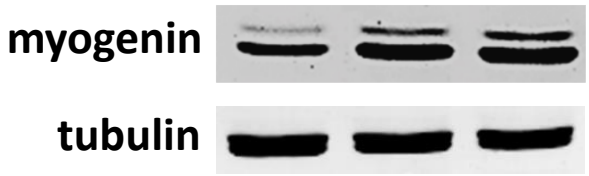
(MHC (myosin heavy chain) and myogenin; muscle differentiation markers)

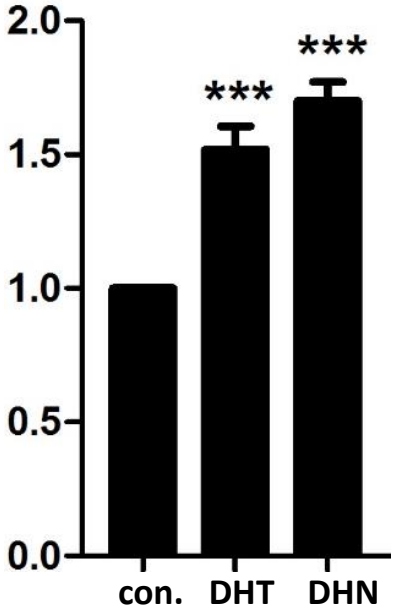
Differentiation

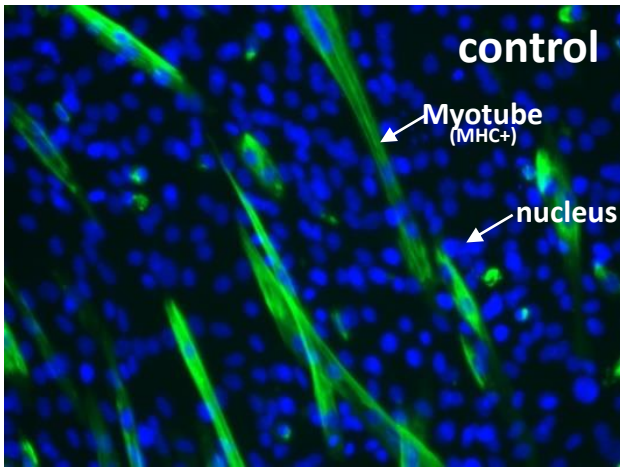
: DHN enhances C2C12 myoblast differentiation

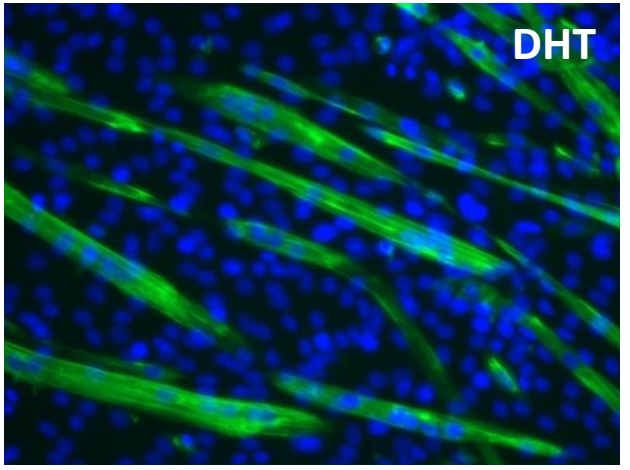


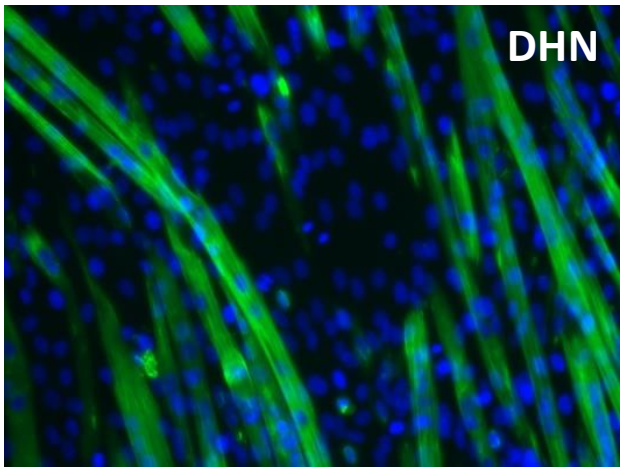






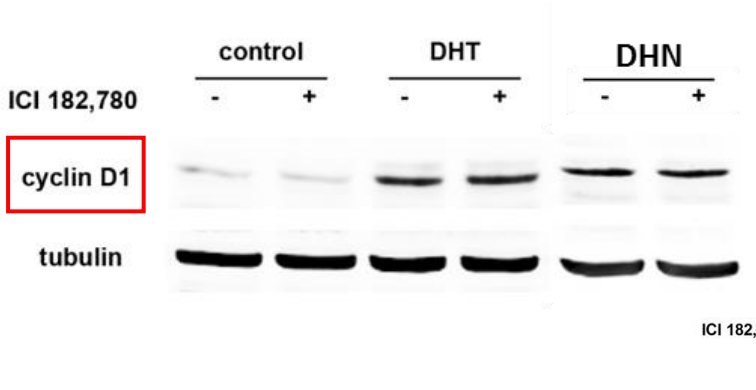


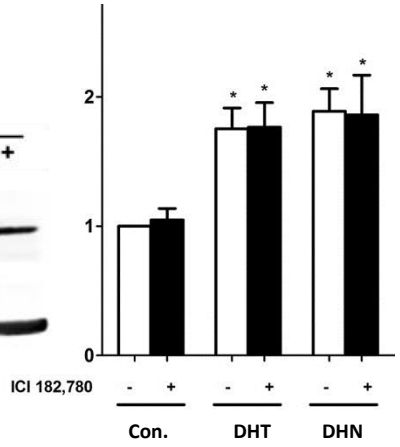


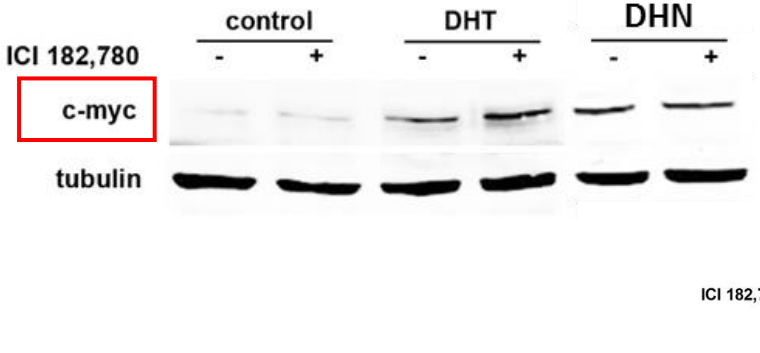


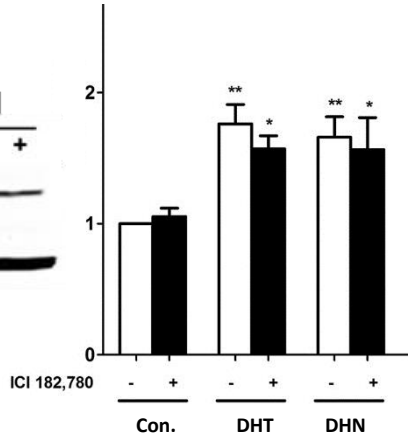
Proliferation

: DHN enhances myoblast proliferation occurred in ER-independent manner

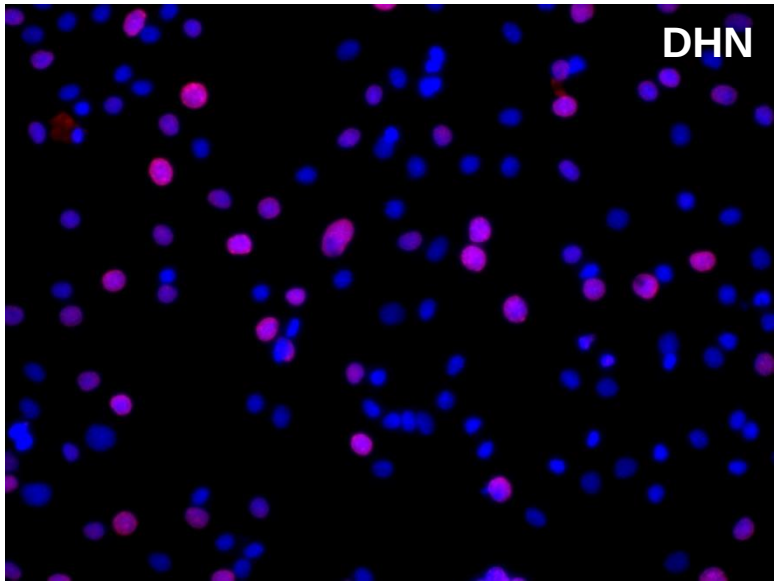


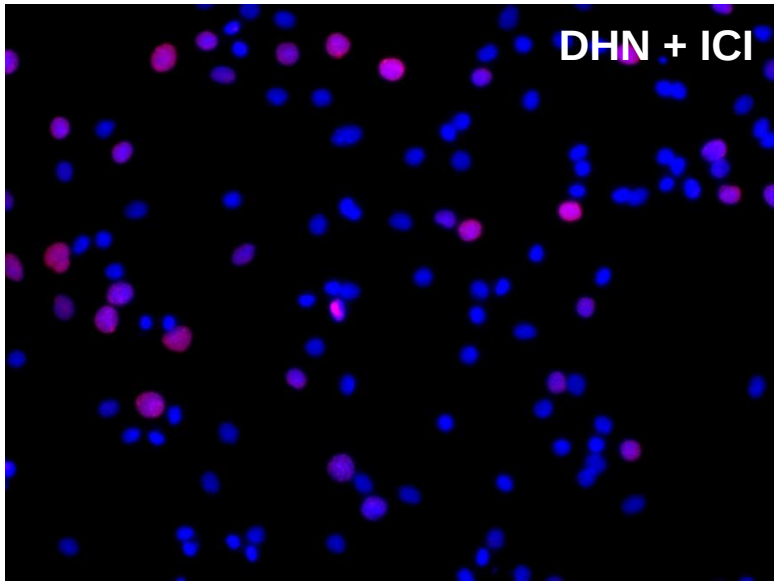






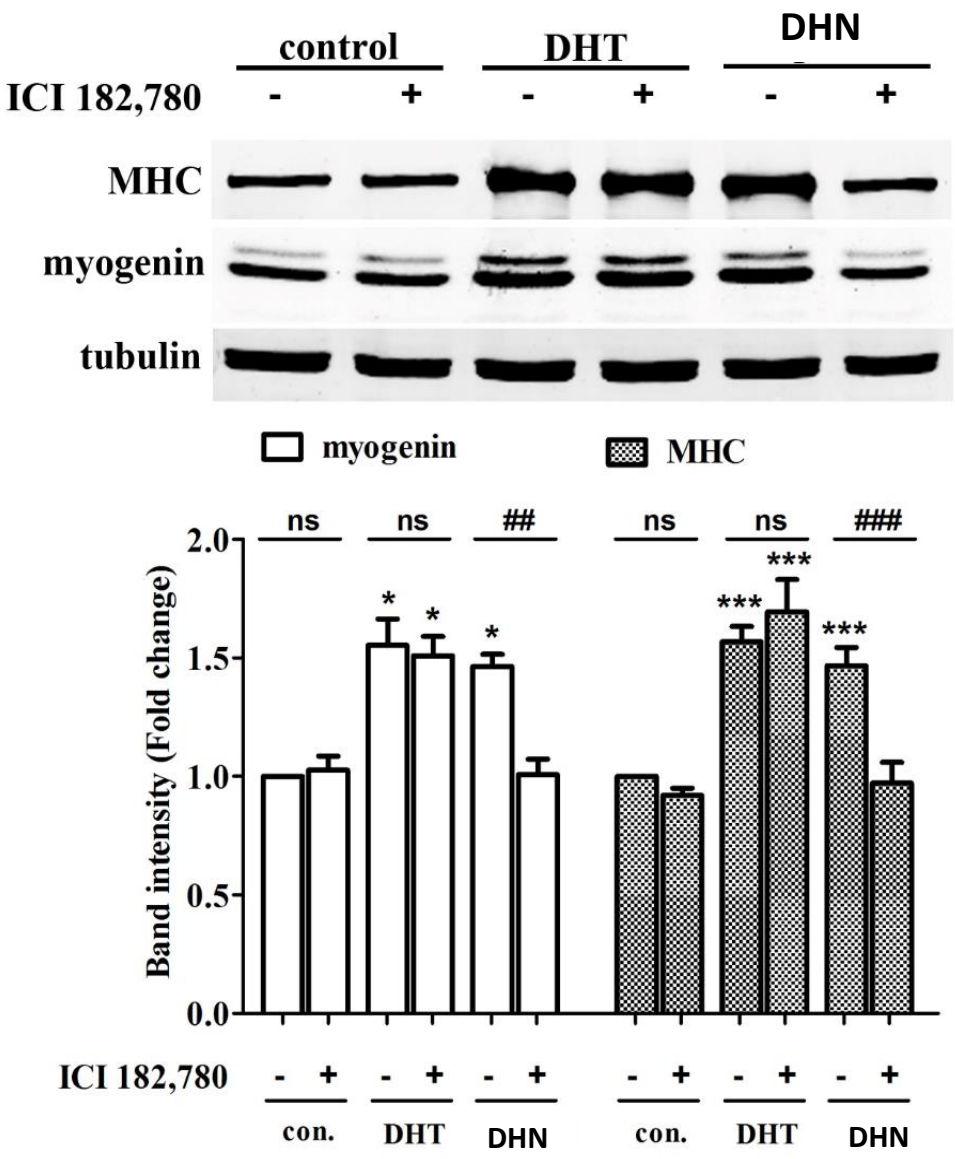
BrdU incorporation assay

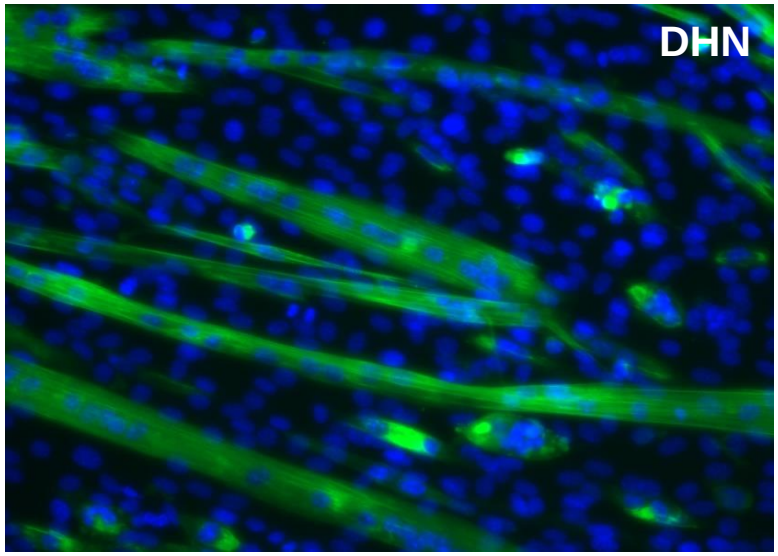


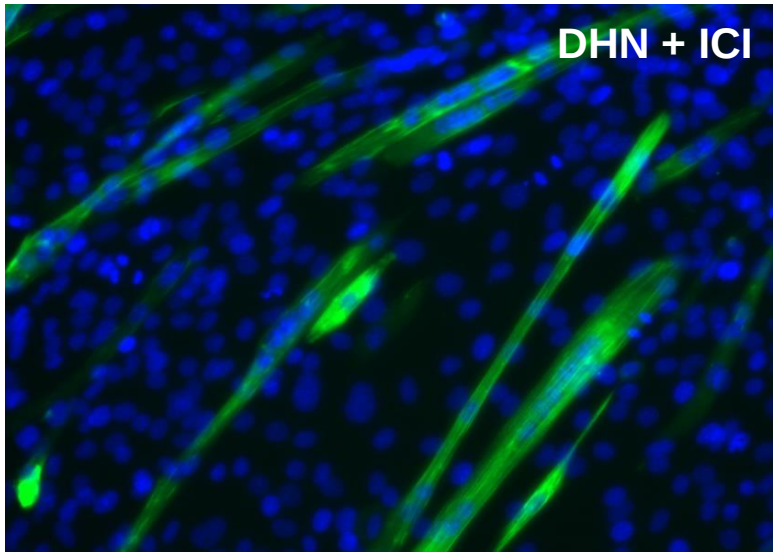


(ER; Estrogen Receptor)

Differentiation : DHN enhances myoblast differentiation in ER-dependent manner



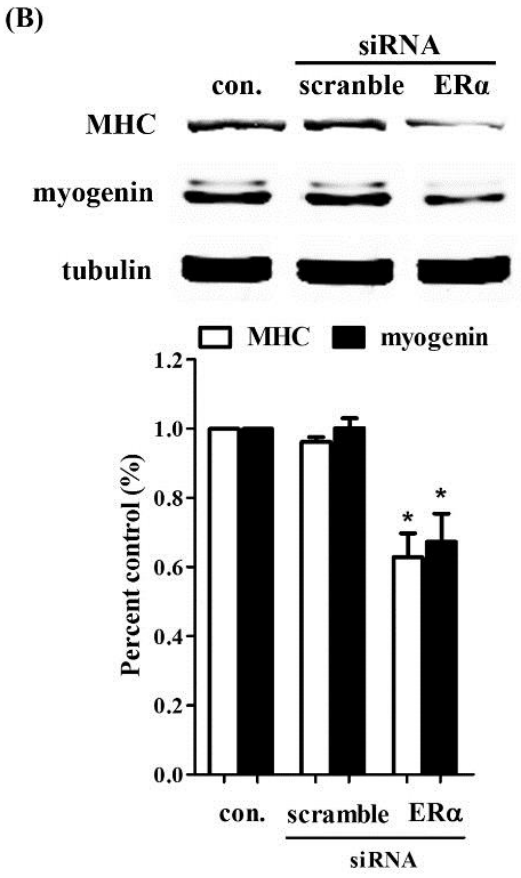




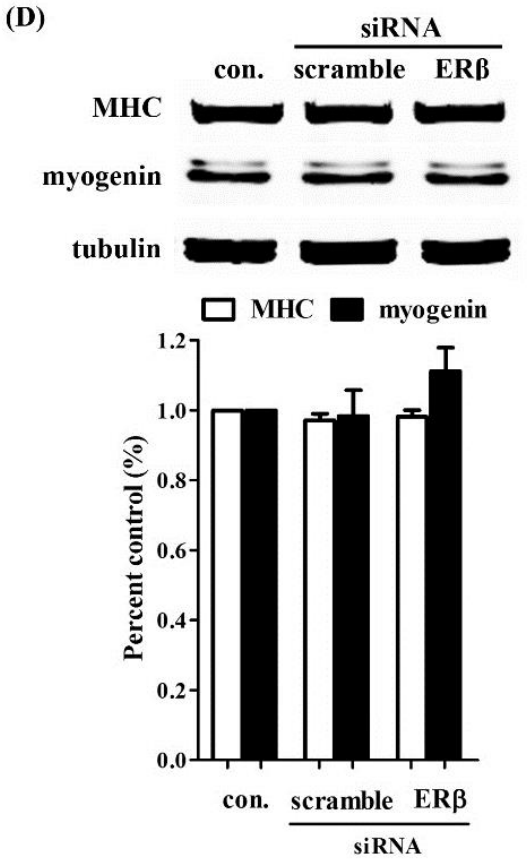
(ICI 182,780; ER inhibitor)

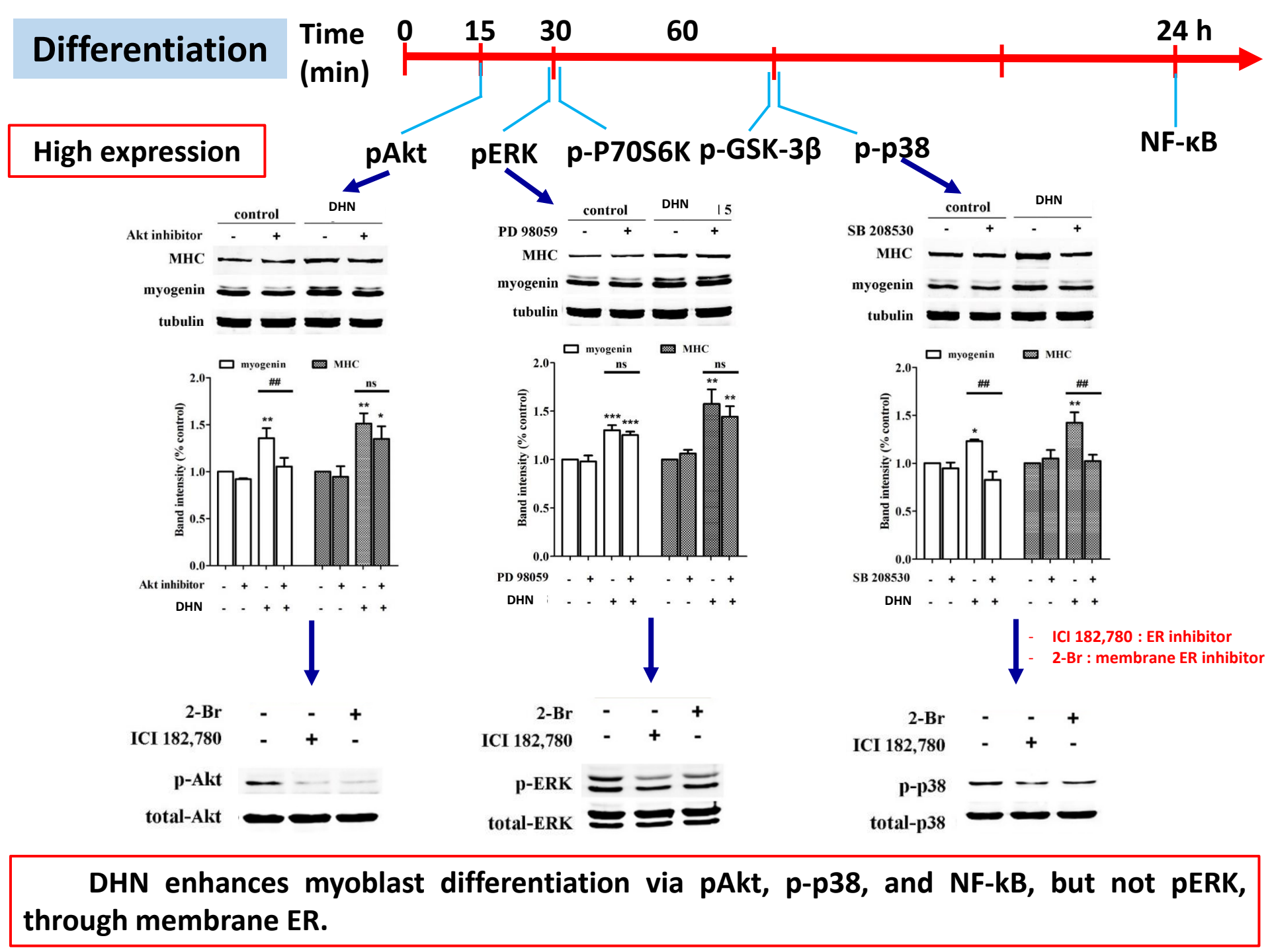
DHN enhances myoblast differentiation through ERα, but not ERβ

ERα siRNA



ERβ siRNA





DHN enhances myoblast differentiation via pAkt, p-p38, and NF-kB, but not pERK, through membrane ER.

Differentiation

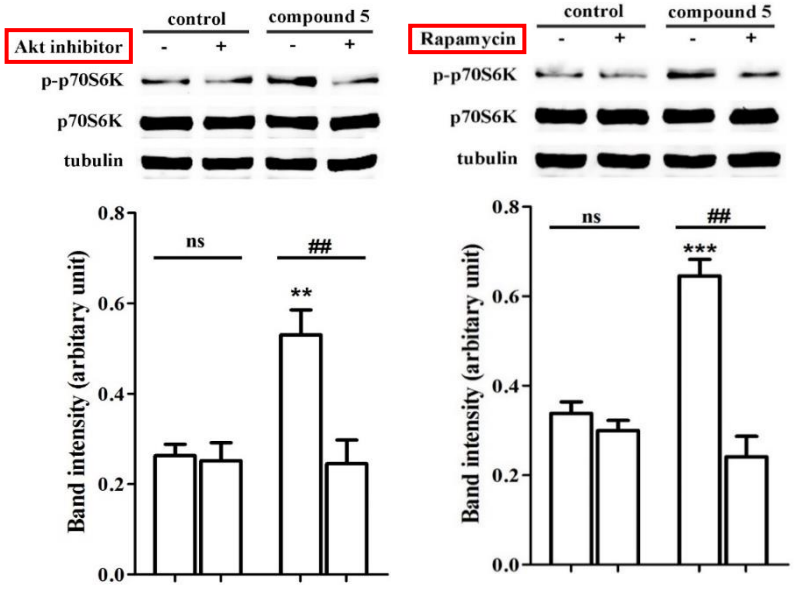
Time (min)

0 15 30 60 24 h

High expression

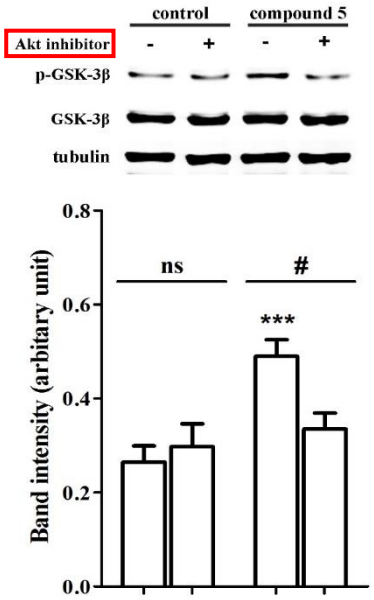
pAkt pERK p-P70S6K p-GSK-3β p-p38 NF-κB

+ Akt inhibitor
+ Rapamycin
+ SB 203580



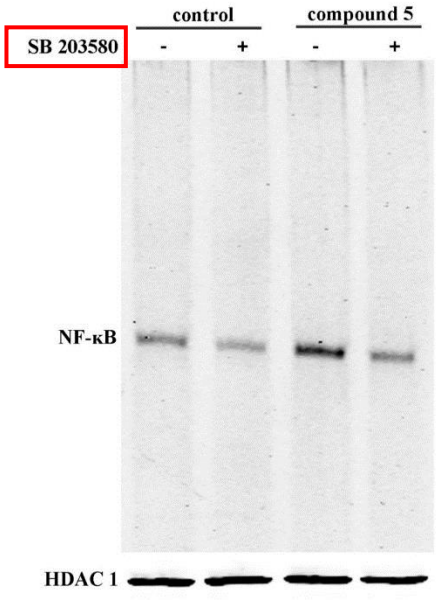
p-P70S6K is a downstream of pAkt/mTOR

+ Akt inhibitor
+ Rapamycin
+ SB 203580



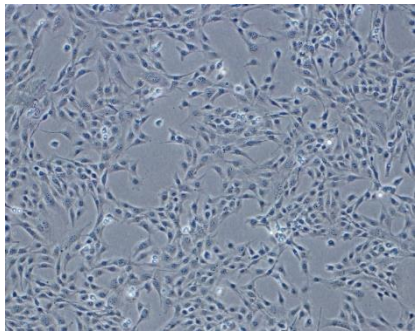
p-GSK-3β is a downstream of pAkt

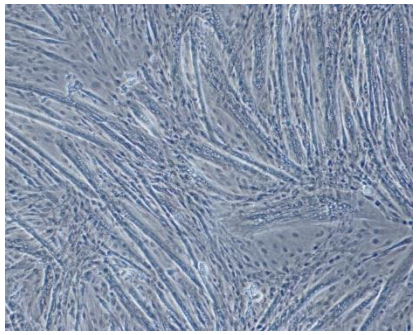
+ Akt inhibitor
+ SB 203580



NF-κB is a downstream of p38

Summary





cell membrane

diarylheptanoid

Cav-1

2-BR

PAT

PA

ICI 182,780

ERα

ERα

Neosynthesized

pAkt

pERK

p38

Protein

- myogenin
- MHC

mTOR

p-GSK-3β

NF-κB

p-p70S6K

- myogenin
- MHC

mRNA

ERα-siRNA

[Cav-1, Caveolin-1; PA, palmitic acid; PAT, palmitoyl-acyl-transferase]

Applications

http://www.bloggang.com/mainblog.php?id=sasiseesom&month=05-07-2012&group=16&gblog=50

This compound may has potential for further development as therapeutic agents for treatment of muscle injury.

The basic knowledge of action between diarylheptanoid and ER, and its downstream signaling pathways in myoblasts may provide a background to develop, modify, and synthesize a better new natural product in the future.

Thank you

for your attention

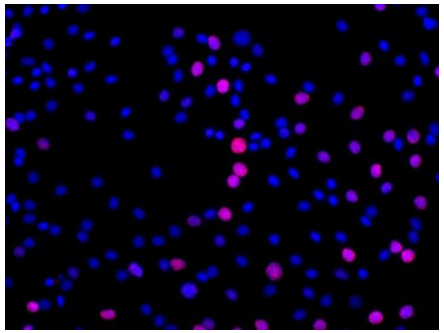
Acknowledgement

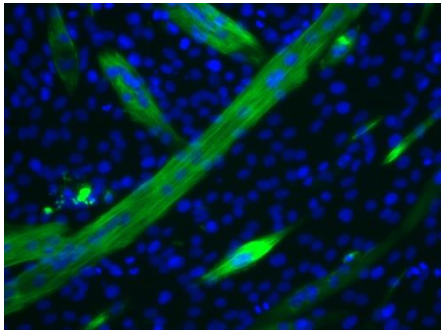
Prof. Yindee Kitiyanant

Prof. Chumpol Pholpramool

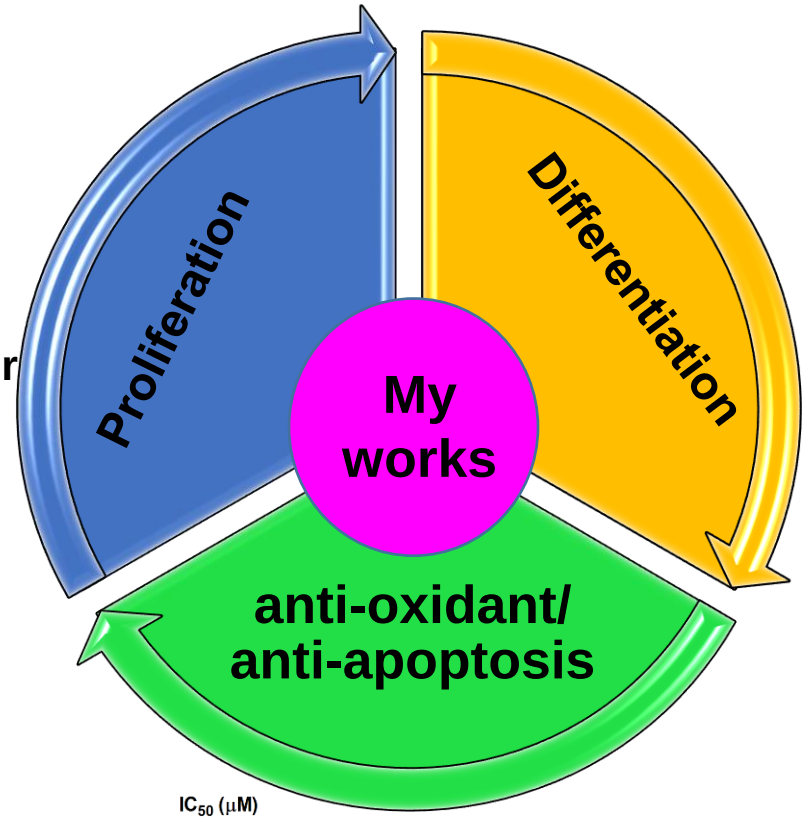
Prof. Karyn A. Esser

This work was supported by the Office of the Higher Education Commission and Mahidol university

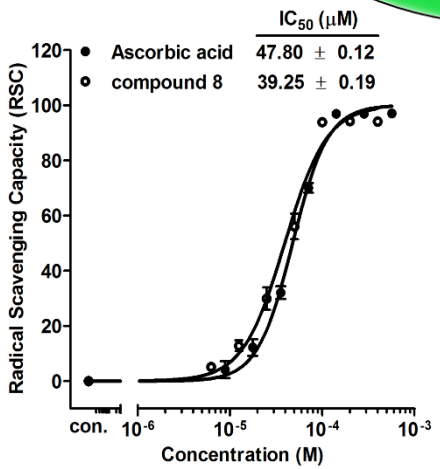




- enhance proliferation
- ER independent manner
- cyclin D1
- c-myc



- enhance differentiation
- ER dependent manner
- specific to ERα subtype
- membrane ER



- IC50 39.25 ± 0.19
- mitigated cell death induced by H_2O_2