# Coordinated time-dependent modulation of AMPK/Akt/mTOR signaling and autophagy controls osteogenic differentiation of human MSC

Pantovic *et al.*
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RESEARCH ARTICLE

# Autophagy mediates cell cycle response by regulating nucleocytoplasmic transport of PAX6 in limbal stem cells under ultraviolet-A stress

**Maria Laggner, Andreas Pollreisz, Gerald Schmidinger, Ursula Schmidt-Erfurth, Ying-Ting Chen***

Department of Ophthalmology & Optometry, Medical University of Vienna, Vienna, Austria

* ying-ting.chen@meduniwien.ac.at



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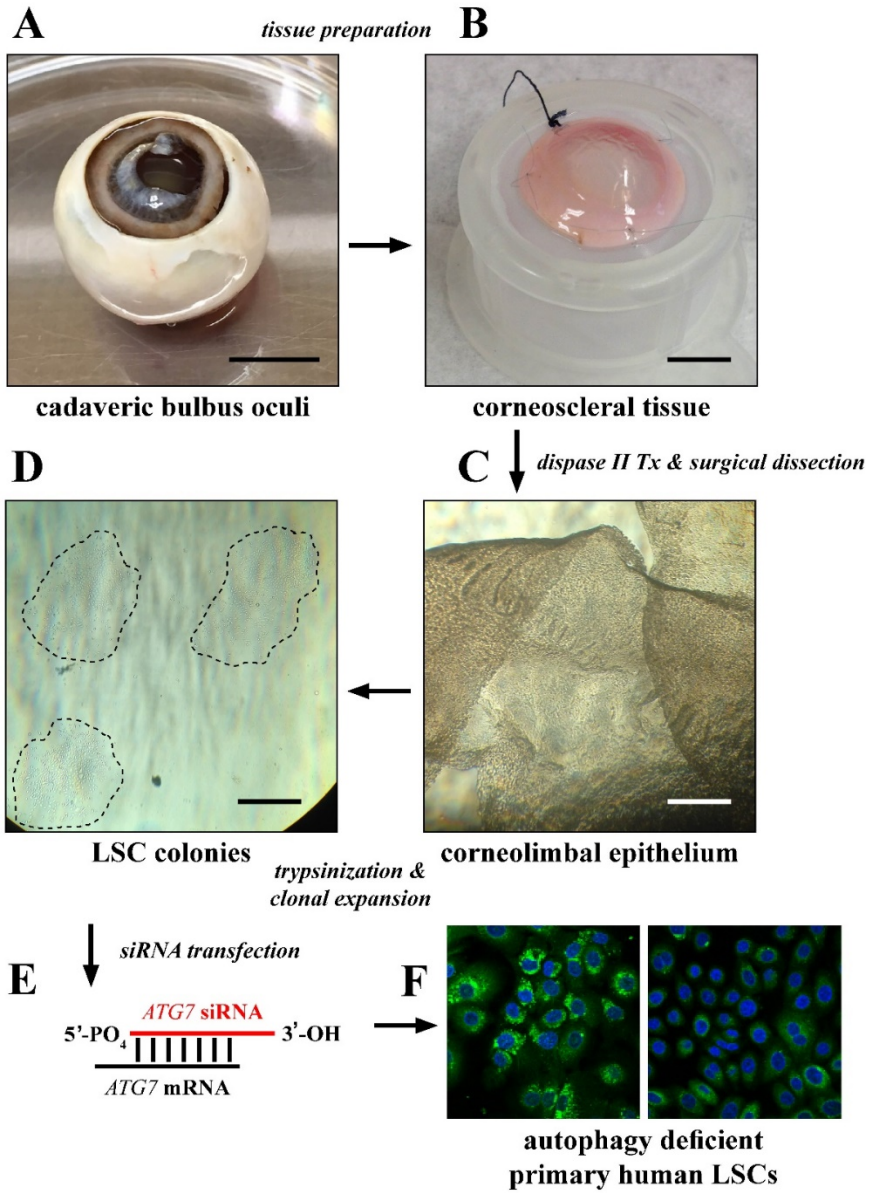
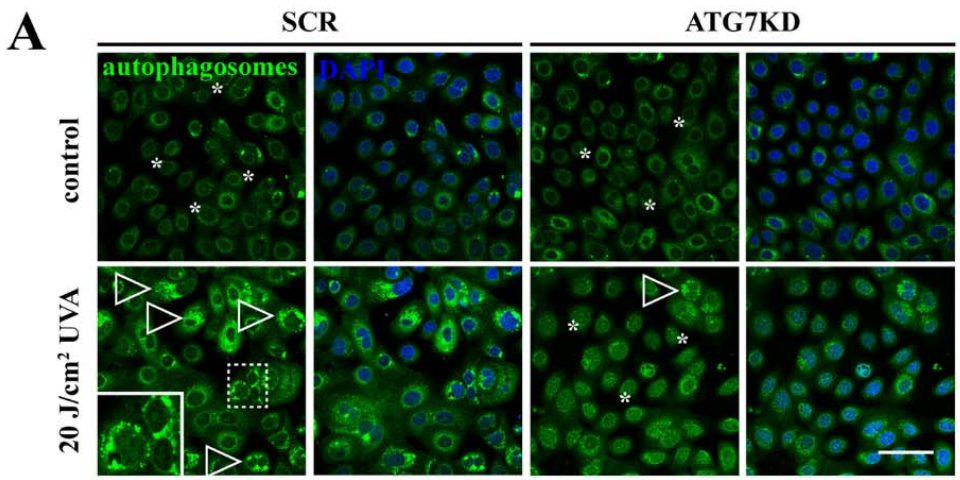
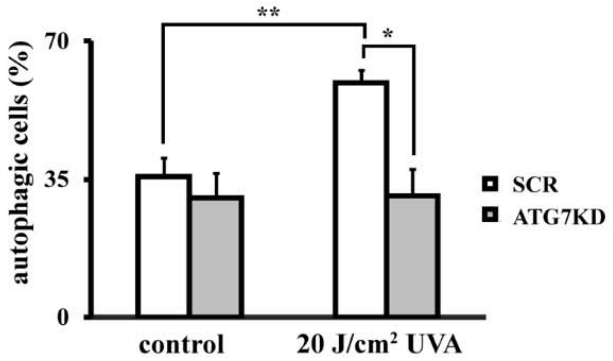
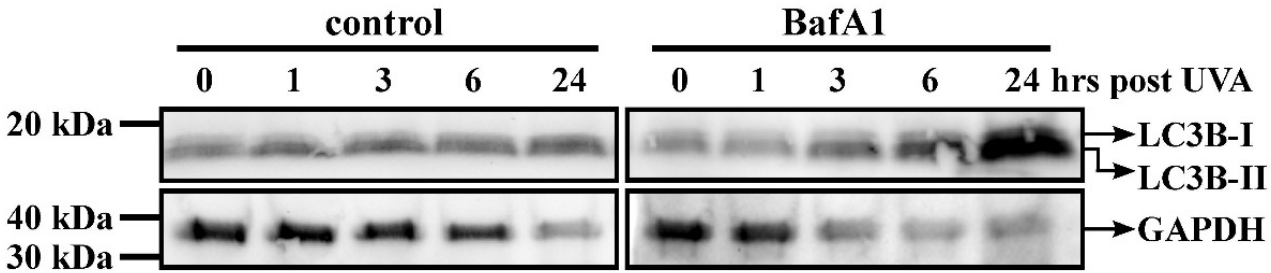
# methodological approach

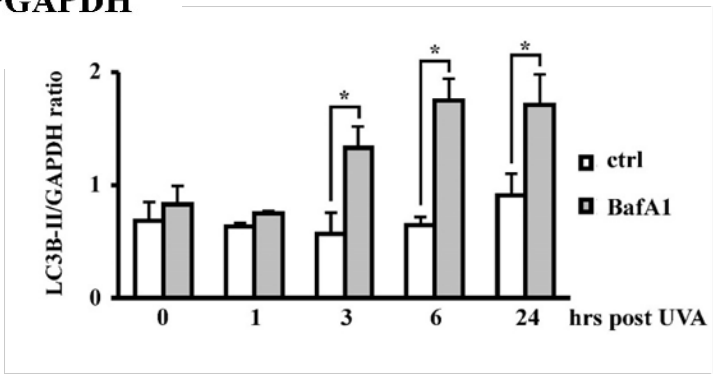


# Autophagy Constitutes LSCs' UVA Stress Response



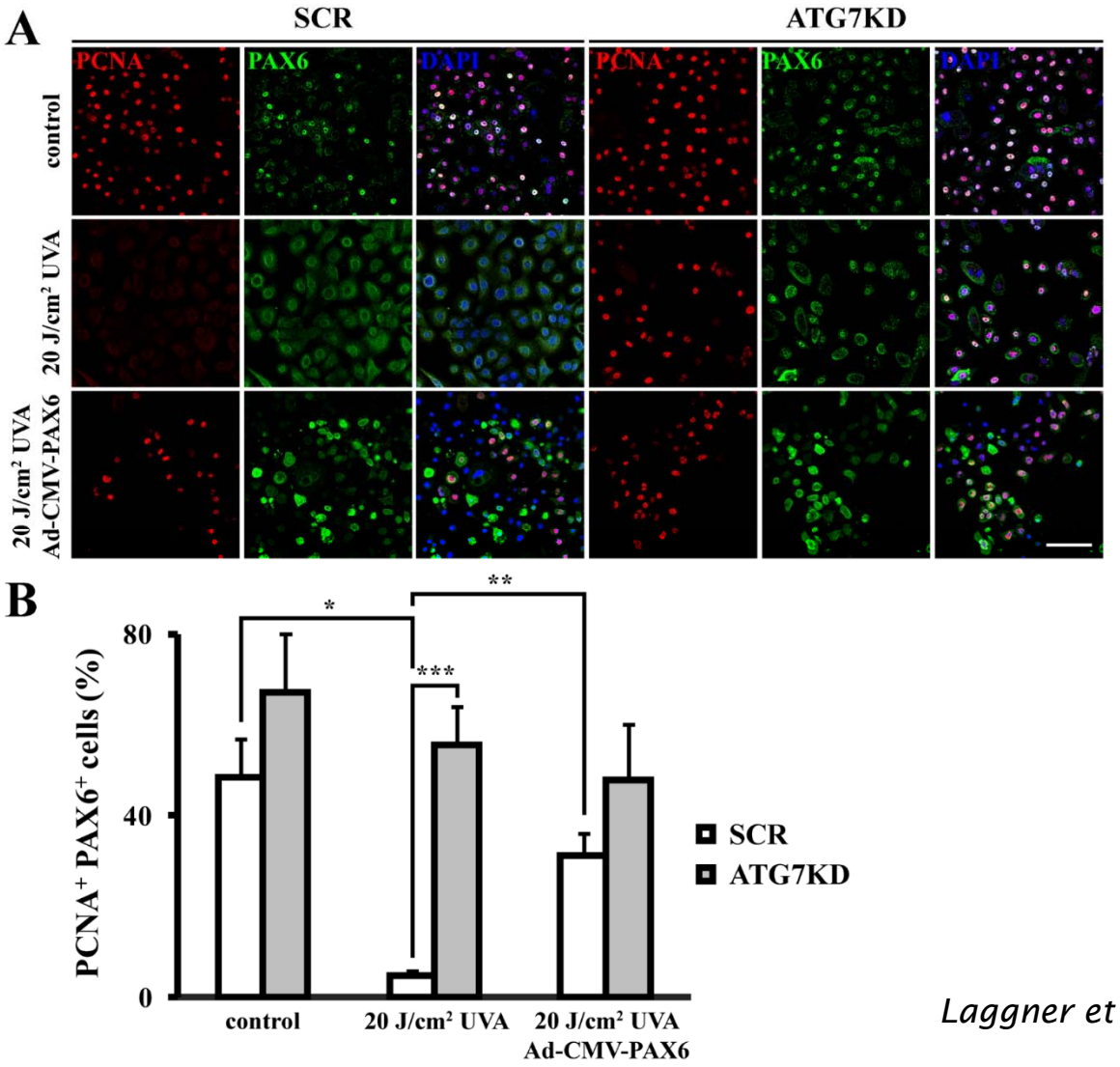




*Laggner et al., PLOS ONE 2017*

# Autophagy dictates PAX6 cytoloccalozation to mediate cell cycle arrest in LSCs under UVA Stress

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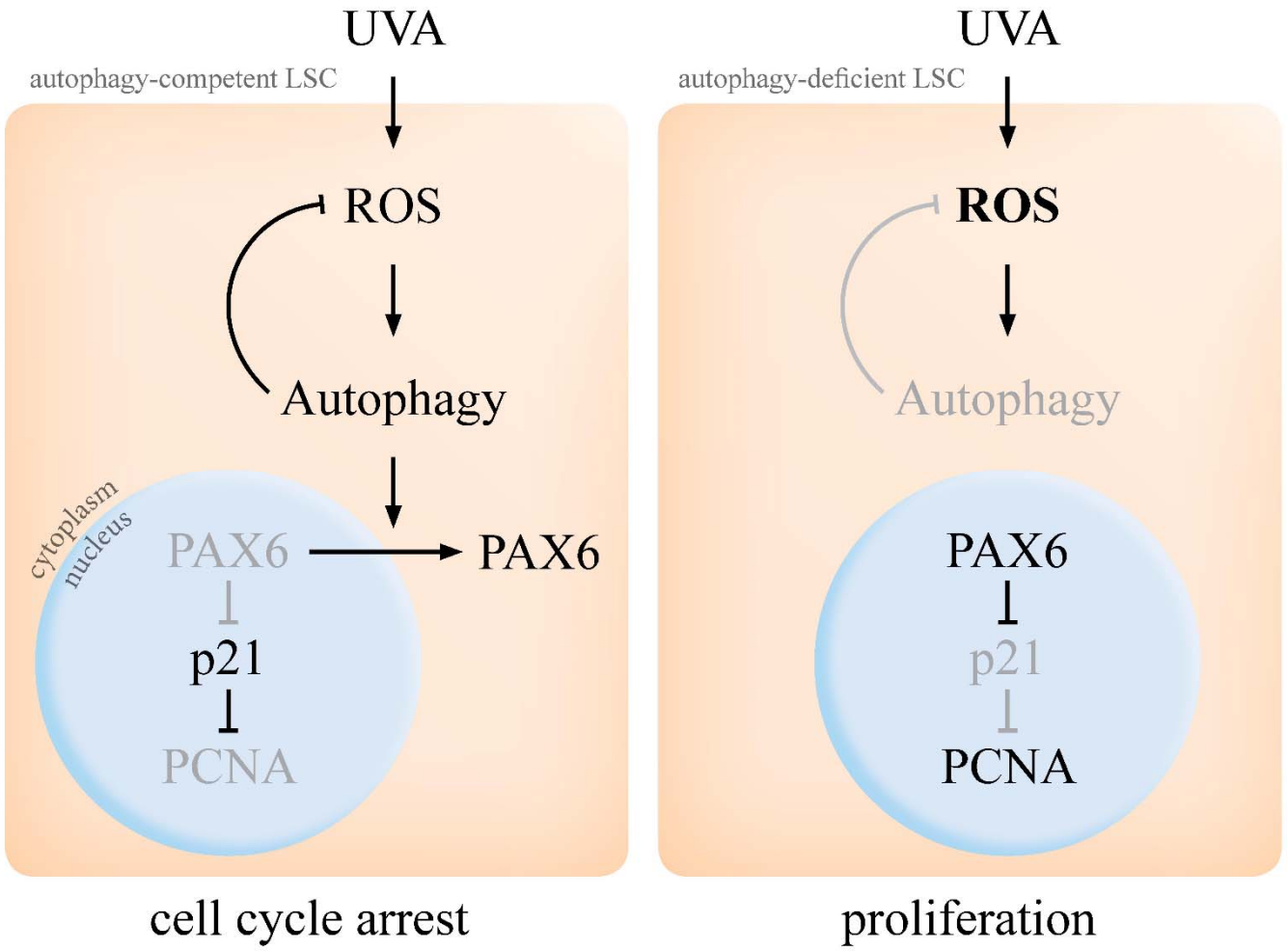
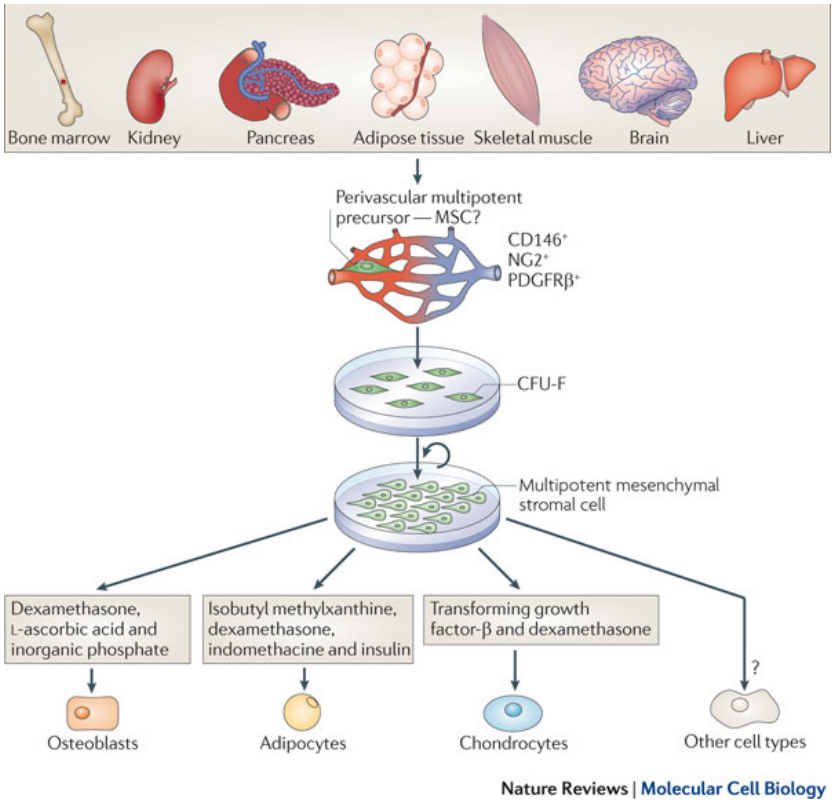
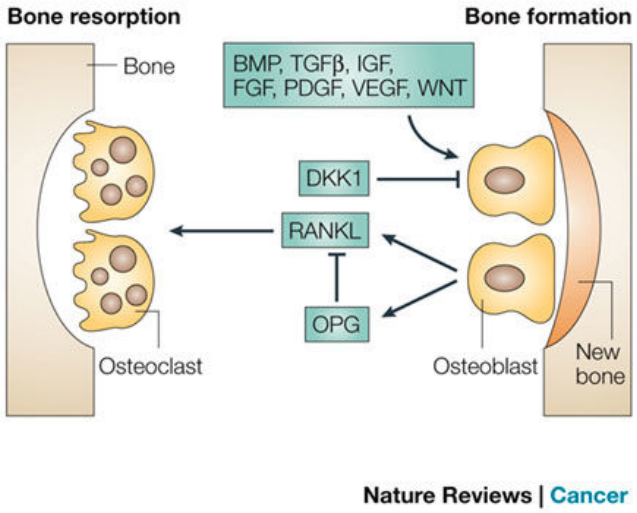



Laggner *et al.*, PLOS ONE 2017

# (dental pulp-derived) mesenchymal stem cells (MSC)




https://www.researchgate.net/profile/Jerome_Ritz/publication/49772528/figure/fig2/AS:267383176691719@1440760525672/MSCs-and-multipotent-mesenchymal-stromal-cellsThe-plastic-adherent-cellular-fraction-of.png

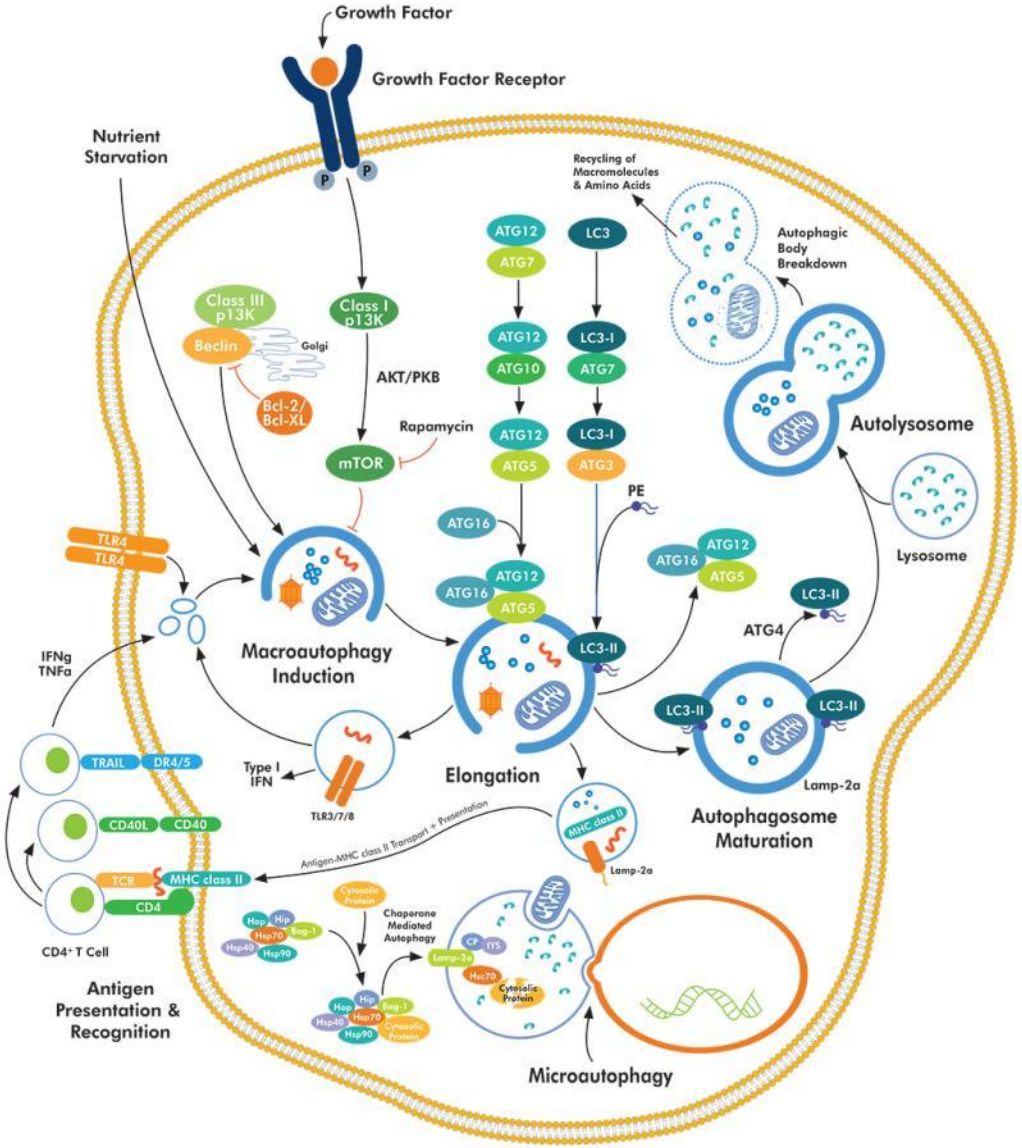



https://media.nature.com/m685/nature-assets/nrc/journal/v5/n1/images/nrc1528-f1.jpg




http://dx.doi.org/10.1016/j.bone.2012.10.024

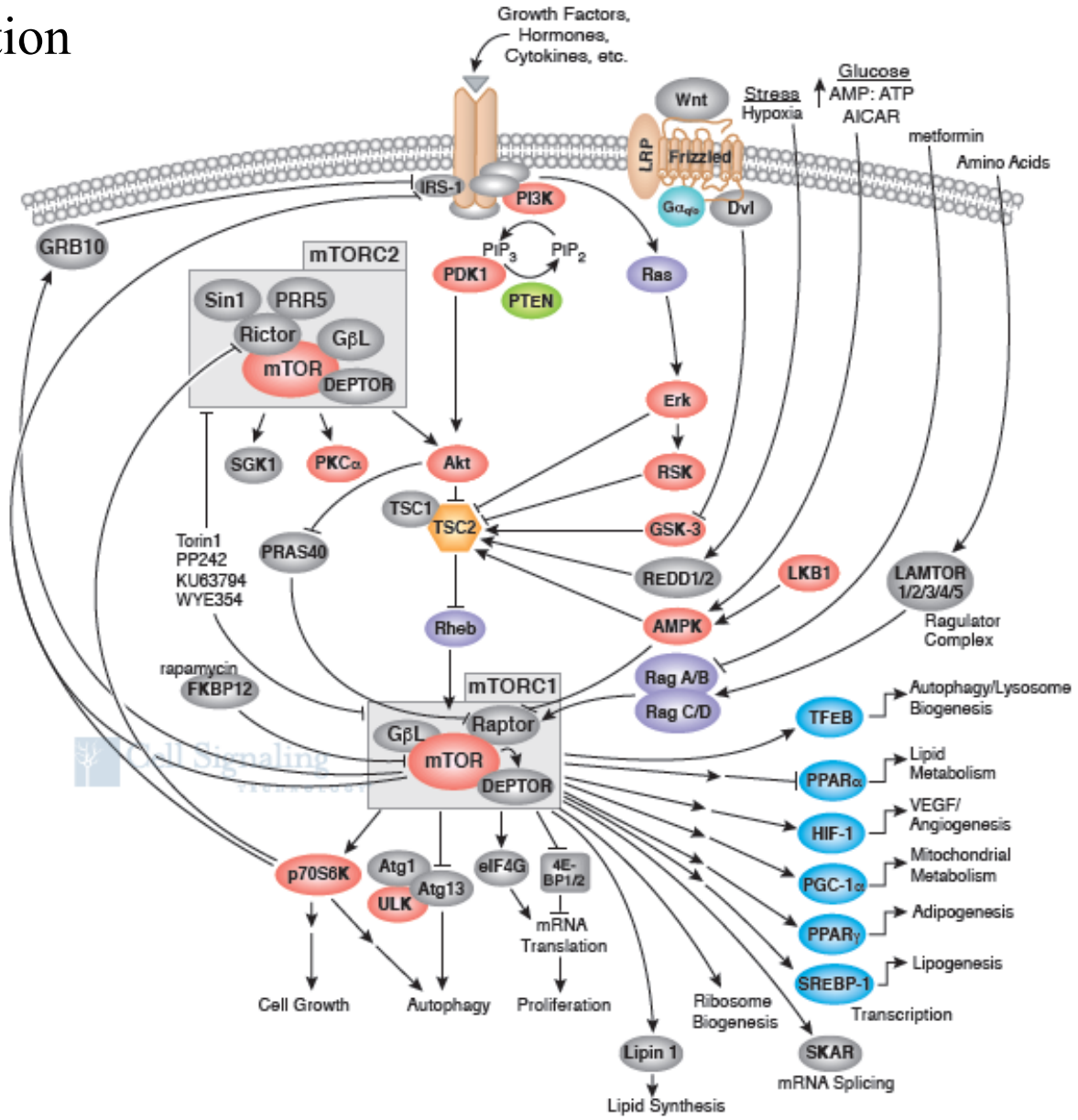
# Autophagy




http://www.novusbio.com/autophagy-pathway.html

# autophagy induction

cellular metabolism
proliferation
survival
death
*differentiation*



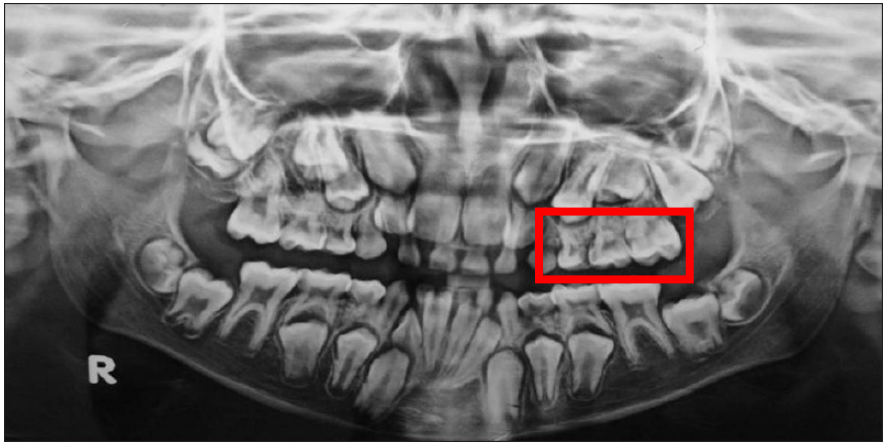
## objective of this study

potential role(s) of aφ & AMPK/Akt/mTOR signaling in osteogenic differentiation of hDP-MSC



http://dx.doi.org/10.1016/j.bone.2012.10.024

# M & M‘s




http://www.jisppd.com/articles/2011/29/4/images/JIndianSocPedodPrevDent_2011_29_4_315_86378_u6.jpg

deciduous teeth
collagenase type I digestion
DP-MSC isolated based on the ablitity to adhere, culture for 3 days
MSC phenotypical verification:
multi-lineage mesenchymal dx/dt ability

osteogenic dx/dt
DMEM/10%FCS + dexamethasone + β-glycerophosphate for 7 d
pharm inh were added at the beginning

marker of osteogenic dx/dt
alkaline phosphatase activity assessed by $OD_{540}$
osteocalcin, BMP2, Runx2



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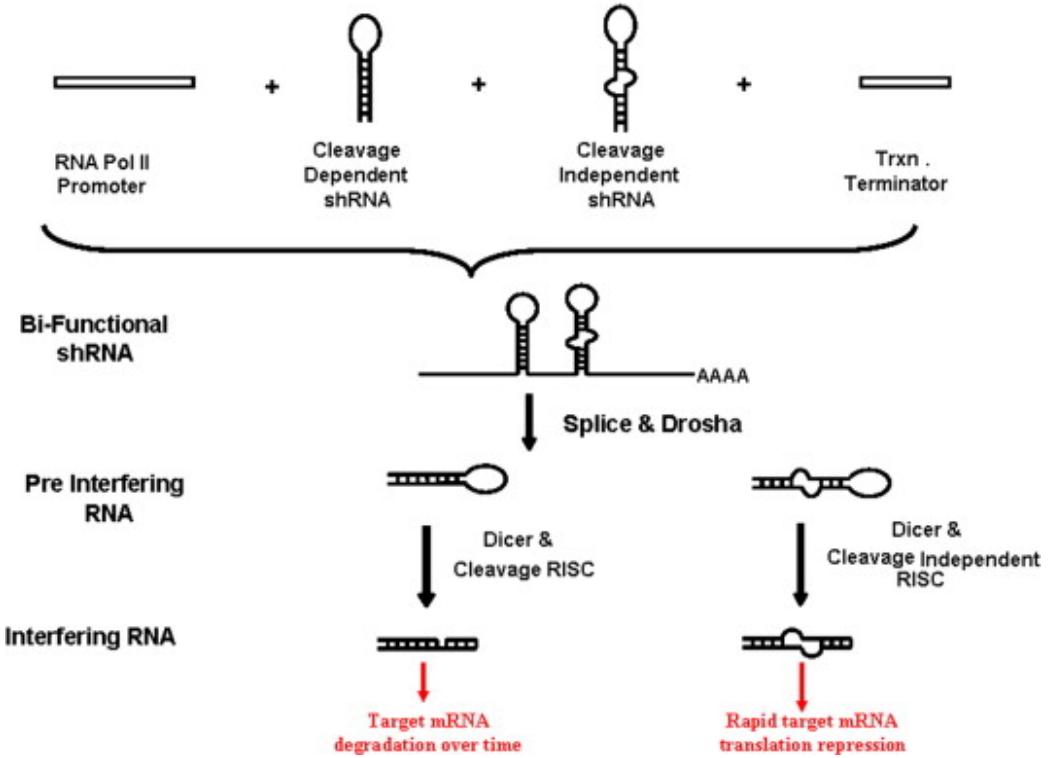
**M & M‘s, c‘d**

pharm inh added on d1

hDP-MSC stably expressing lentiviral vector plasmids encoding human AMPK or LC3 **shRNA**

mTOR **siRNA**

statistical analysis:
student‘s *t* test




https://ars.els-cdn.com/content/image/1-s2.0-S0169409X09000969-gr5.jpg



http://dx.doi.org/10.1016/j.bone.2012.10.024

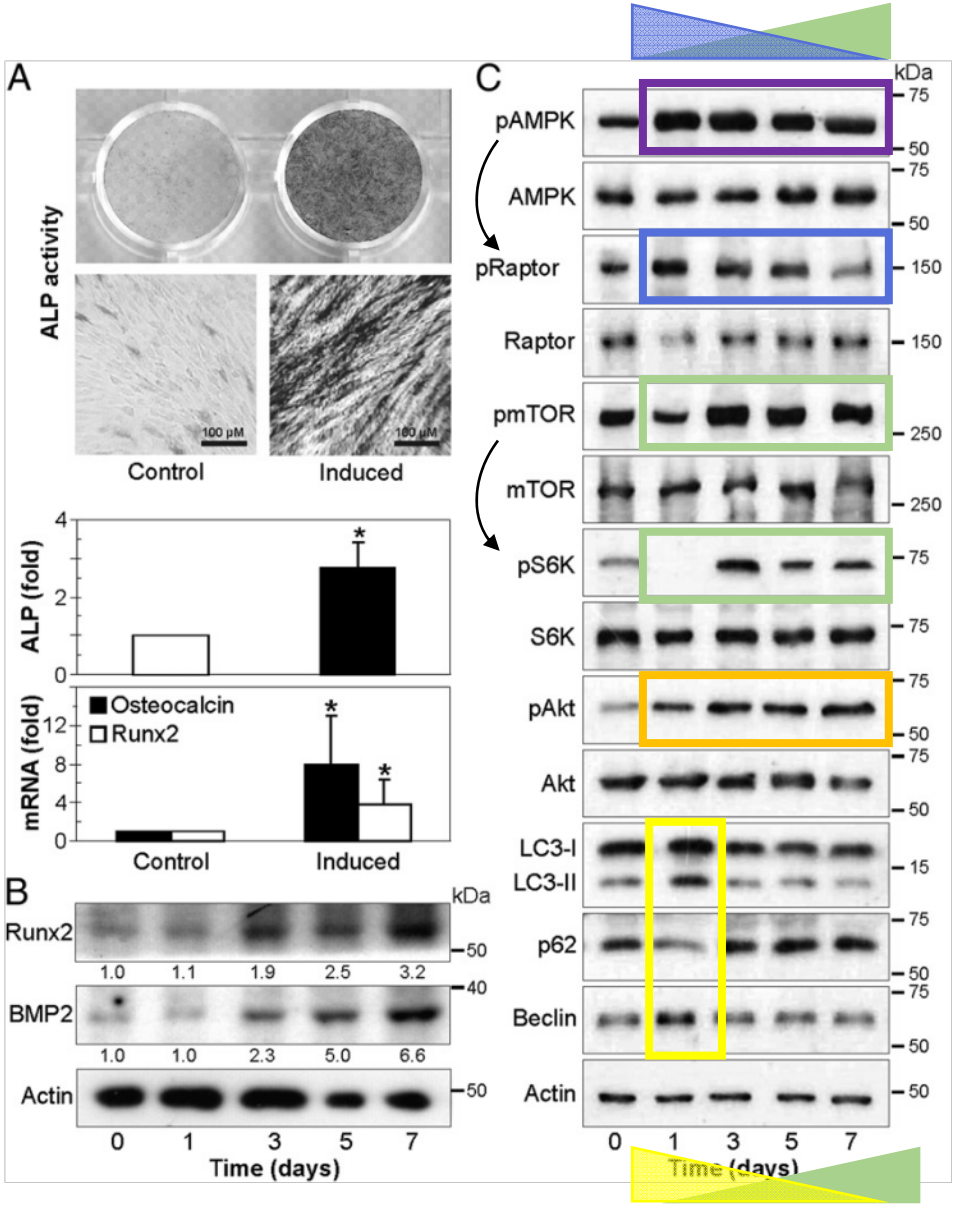
# Results



http://dx.doi.org/10.1016/j.bone.2012.10.024

# early AMPK activation, transient aφ induction & late Akt/mTOR activation

osteogenesis marker







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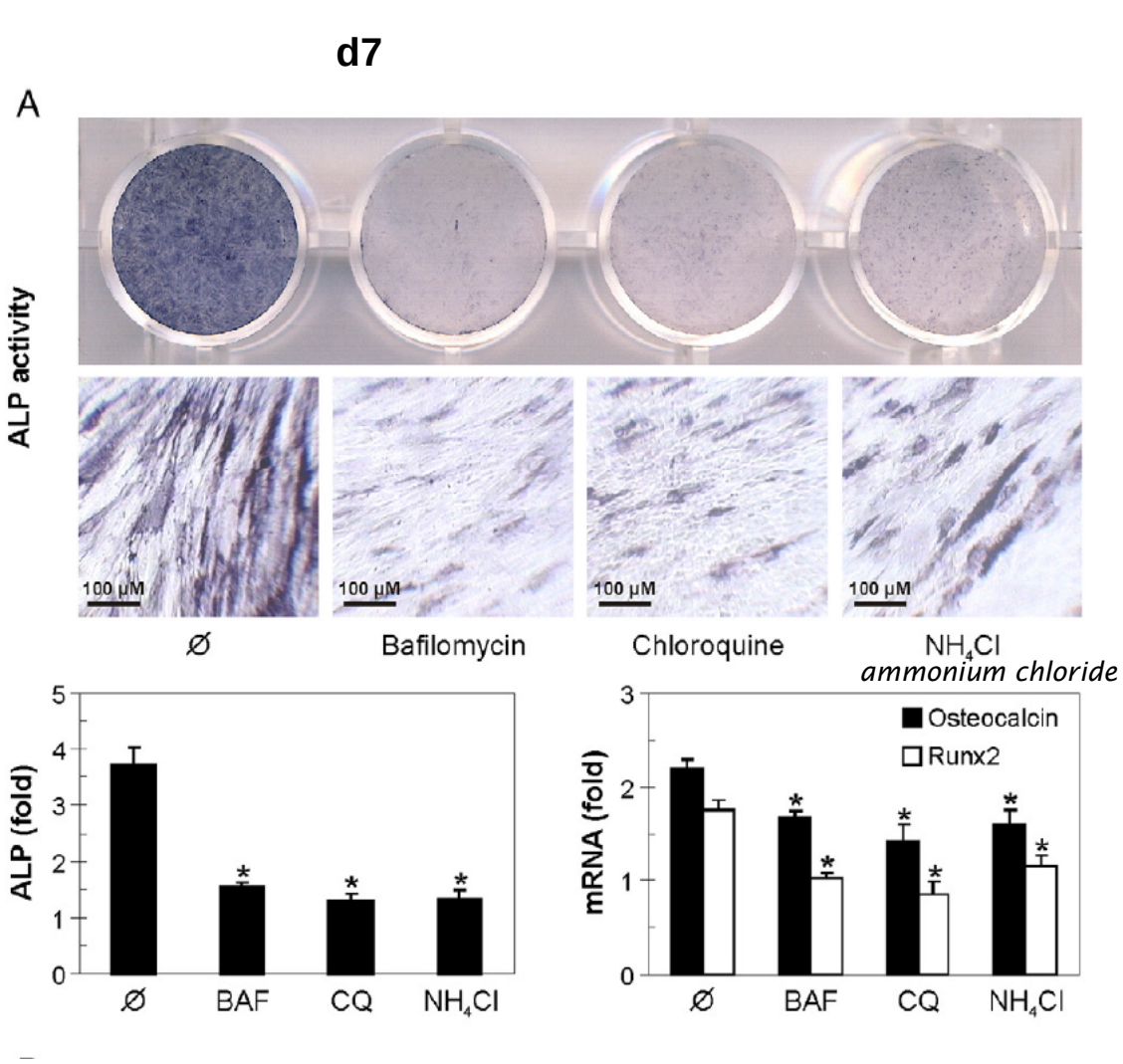
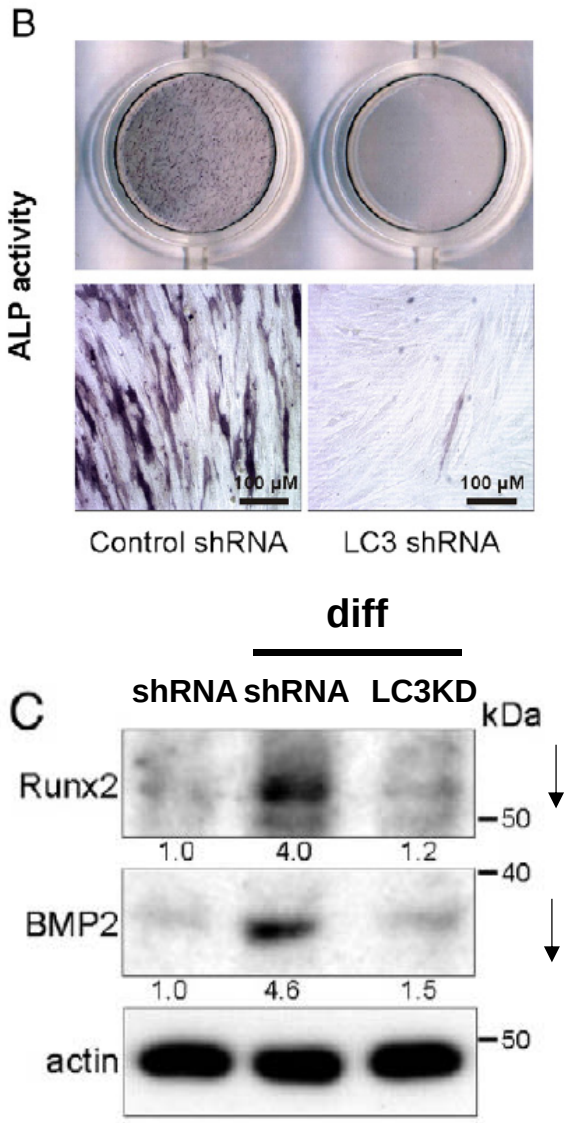
# Autophagy is required for early osteogenic differentiation of hDP-MSC

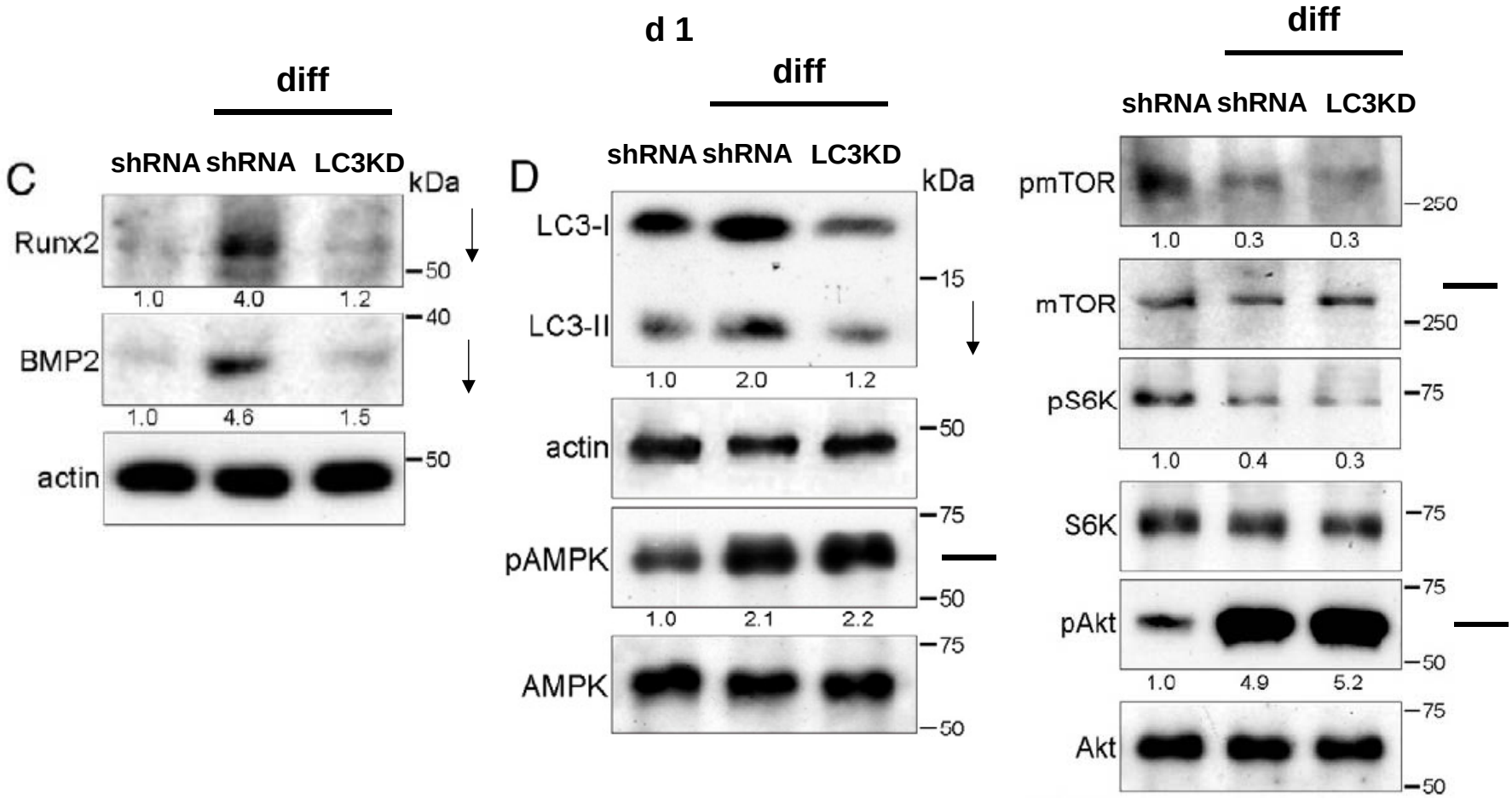





http://dx.doi.org/10.1016/j.bone.2012.10.024

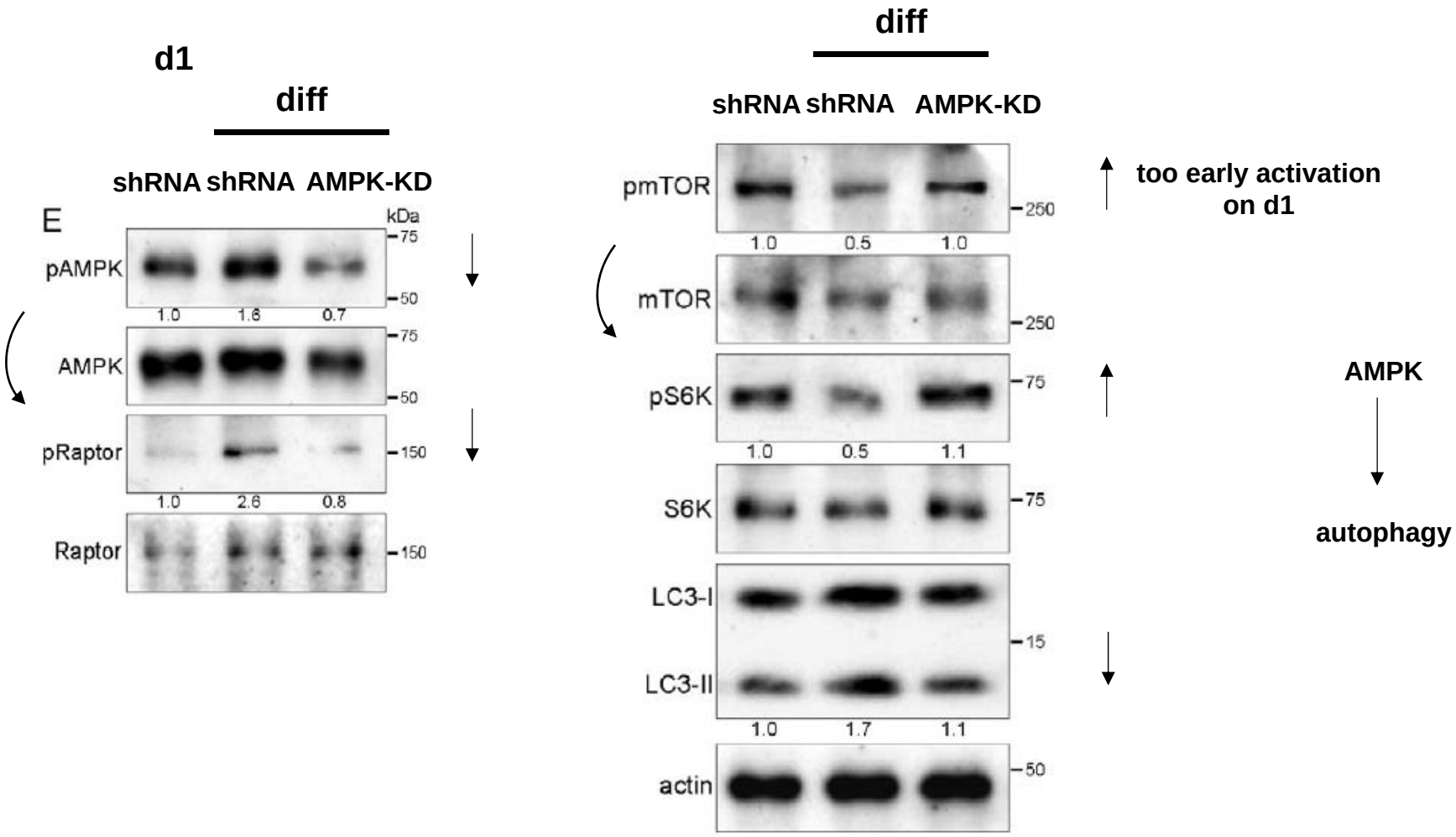
# Autophagy is required for early osteogenic differentiation of hDP-MSC, c'd






http://dx.doi.org/10.1016/j.bone.2012.10.024

# early AMPK-dependent aφ is required for osteogenic MSC differentiation

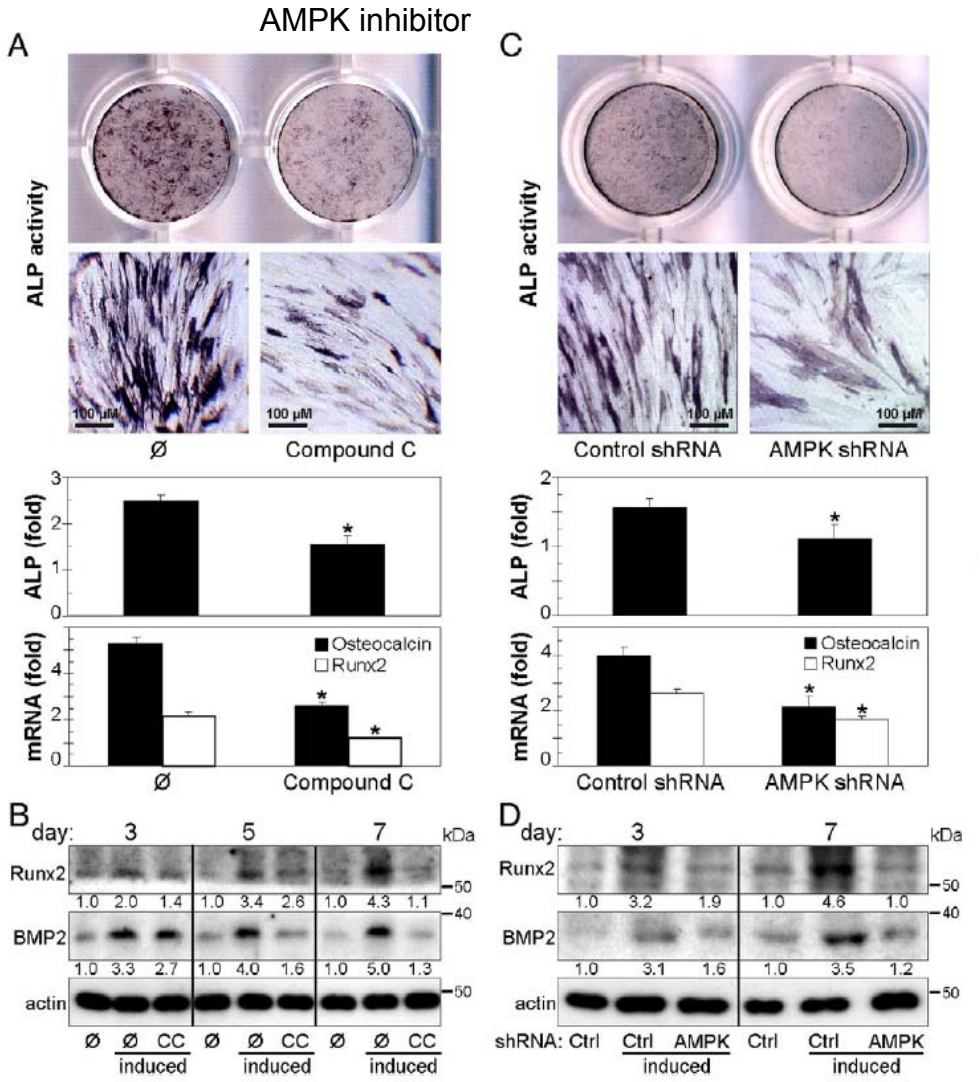





http://dx.doi.org/10.1016/j.bone.2012.10.024

# AMPK-dependent aφ is required for osteogenic MSC differentiation

**d7**




**addition on d3**
**compound C**
**BafA1 or CQ**
**failed to -| dx/dt**

**AMPK**

↓

**autophagy**

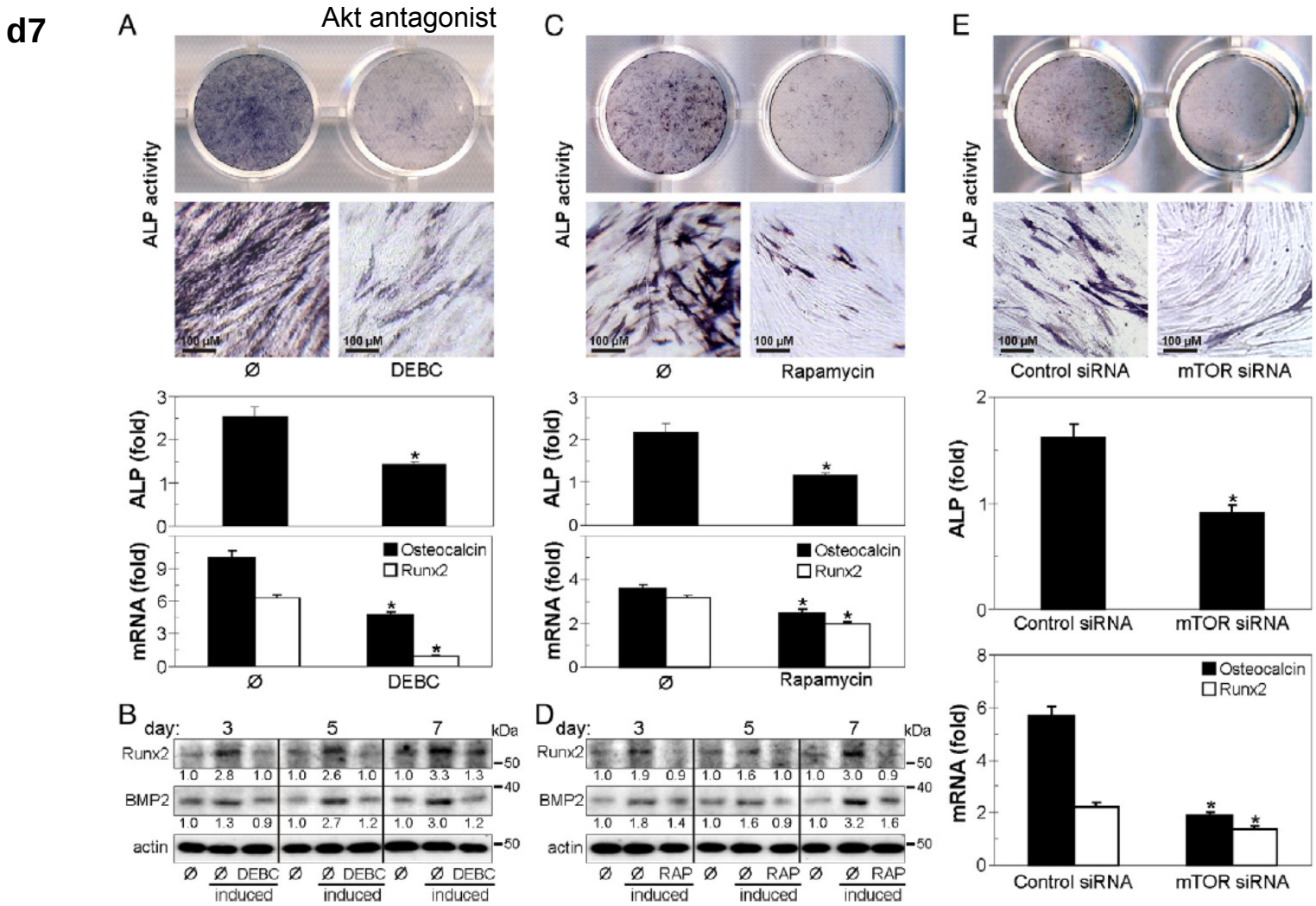
↓

**osteogenic diff**




http://dx.doi.org/10.1016/j.bone.2012.10.024

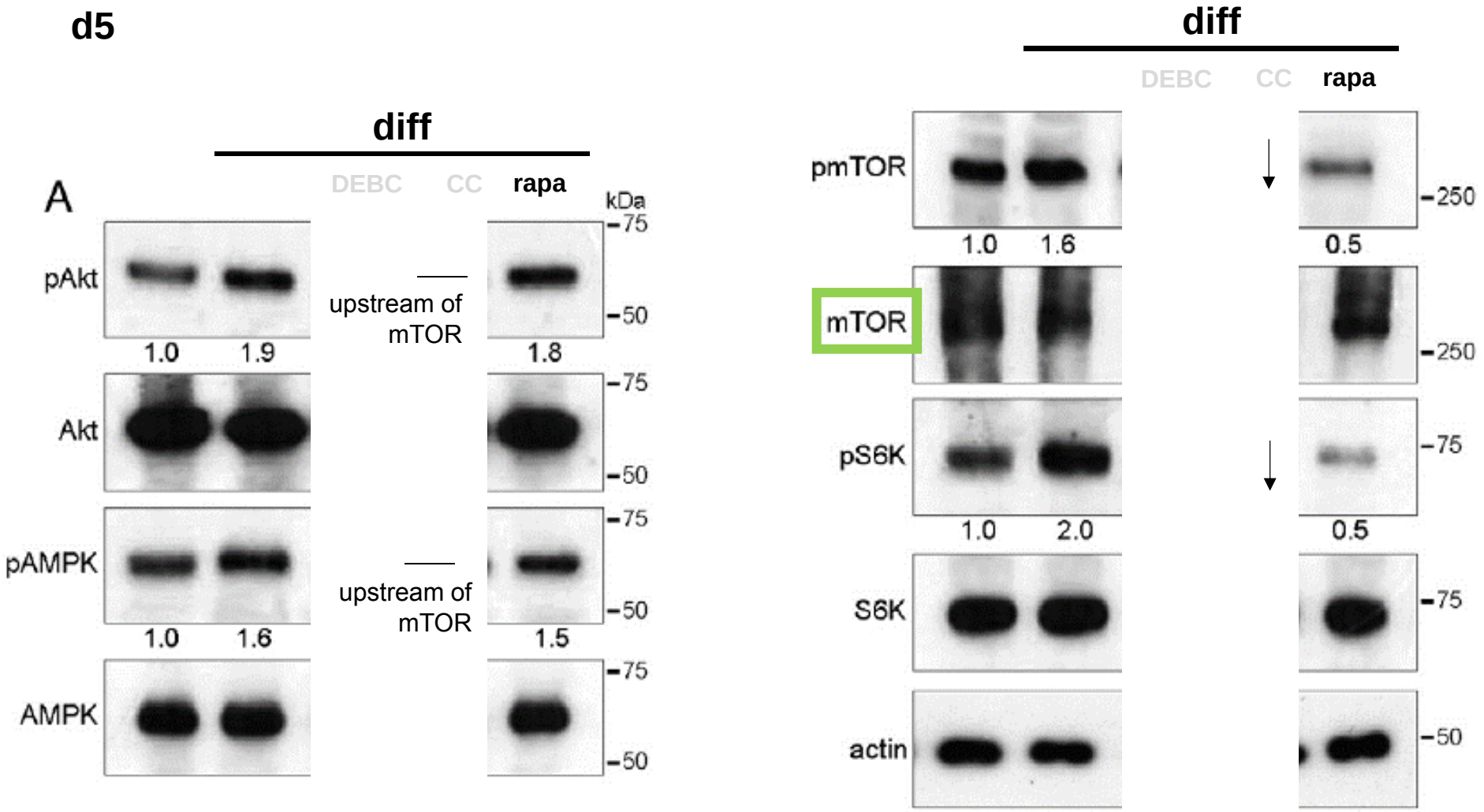
# Akt-dependent activation of mTOR is required for osteogenic differentiation of hDP-MSC






http://dx.doi.org/10.1016/j.bone.2012.10.024

# Akt/mTOR-dependent osteogenic differentiation of hDP-MSC is mediated by AMPK







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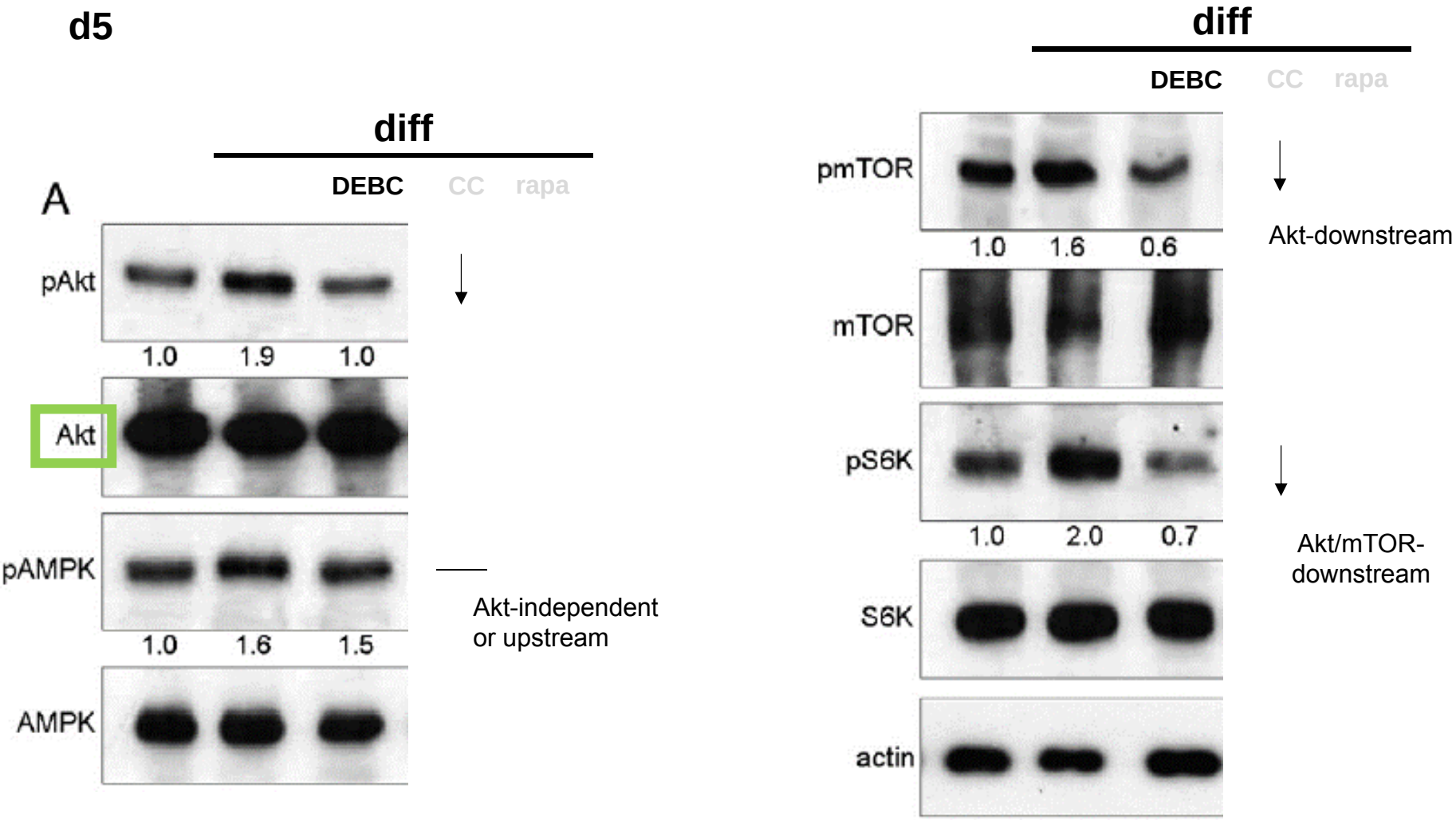





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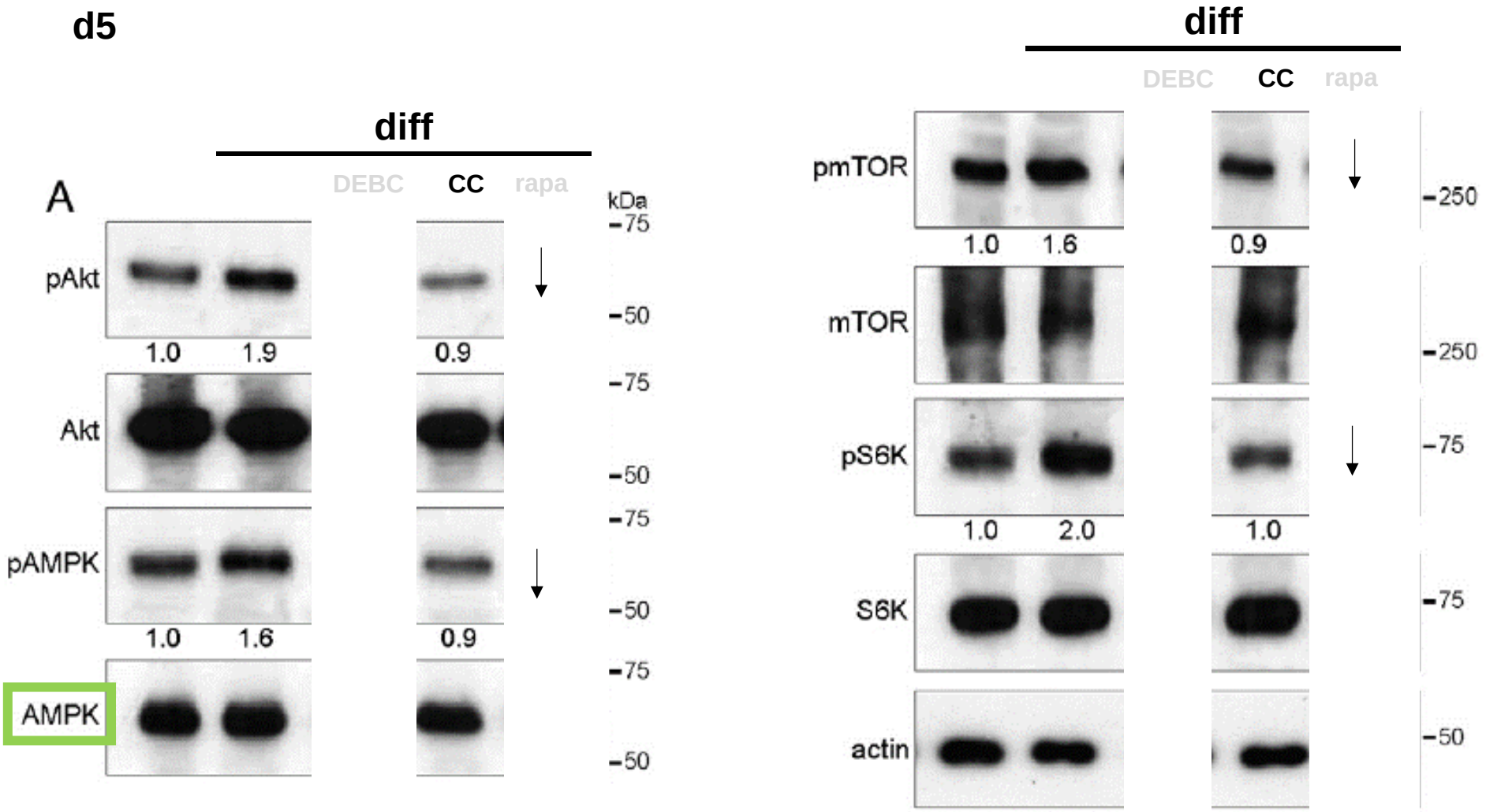





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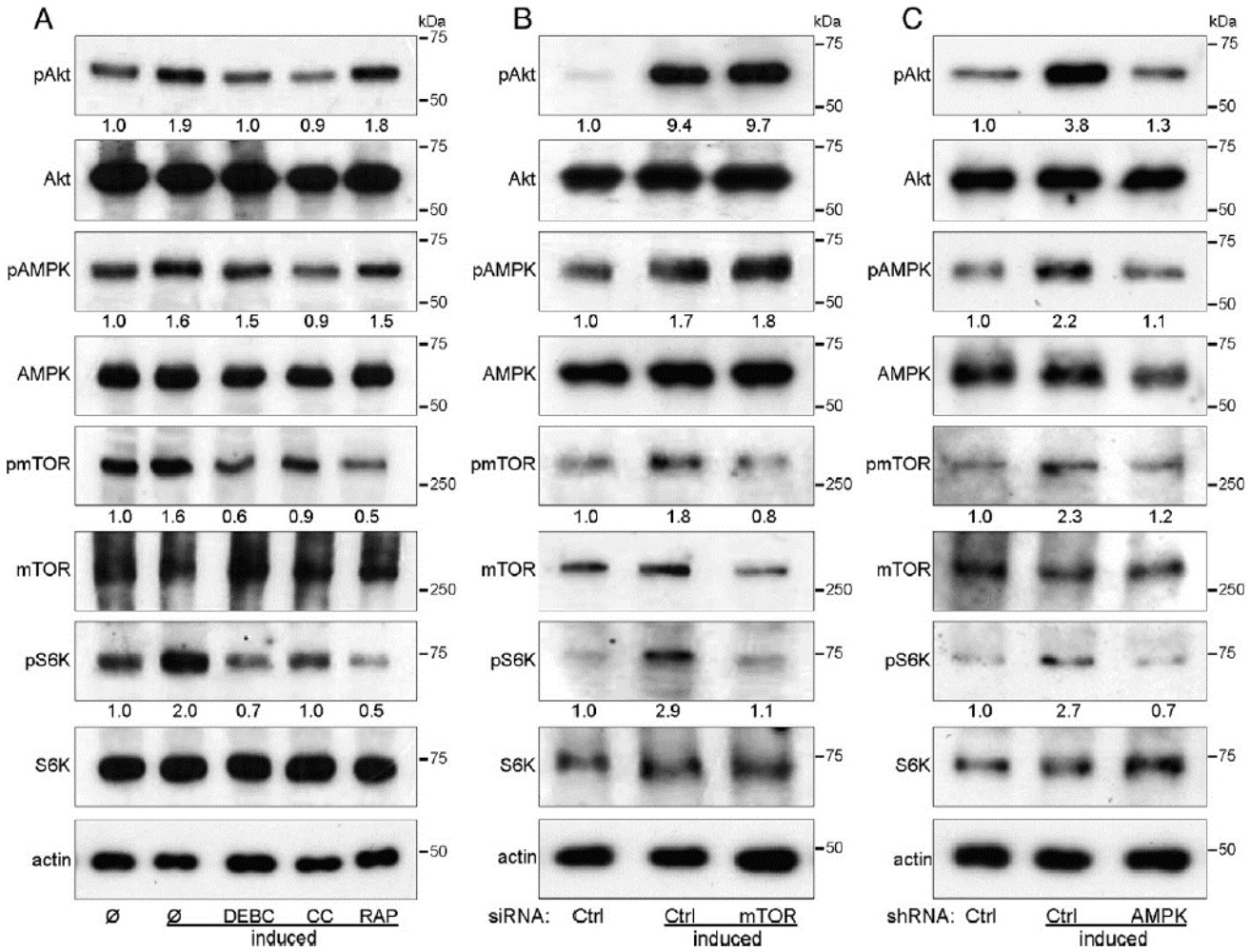
# Akt/mTOR-dependent osteogenic differentiation of hDP-MSC is mediated by AMPK







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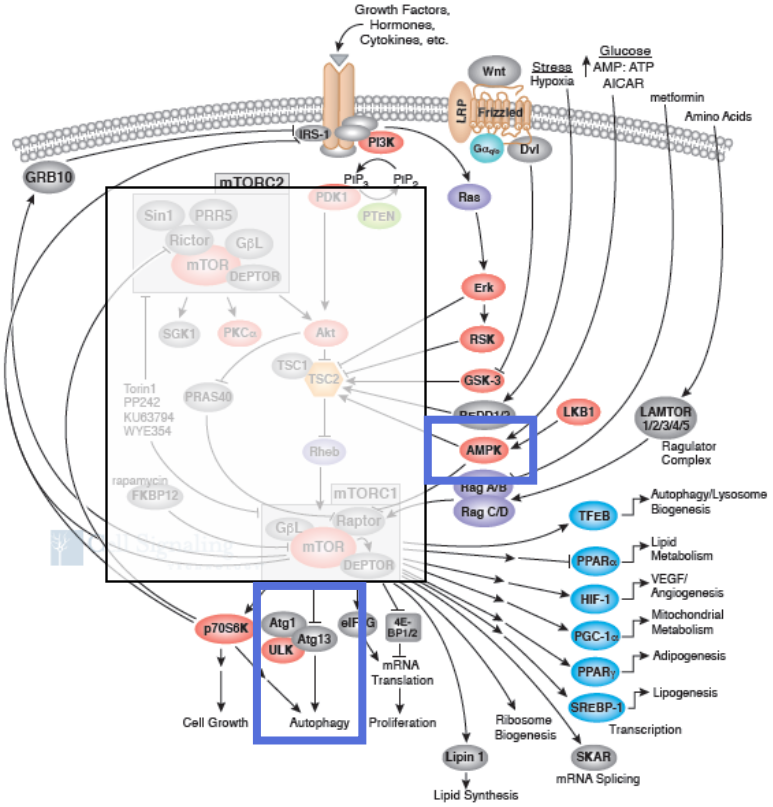
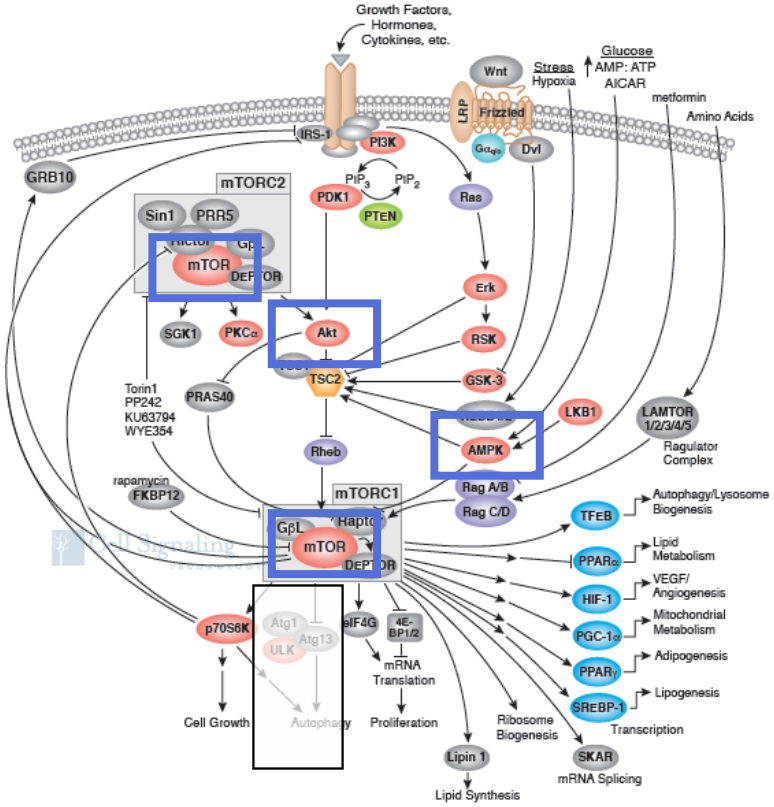
early diff stage:
AMPK (Raptor) -| mTOR -| autophagy

late stage:
AMPK -> Akt-P -> **mTOR** (S6K) -| autophagy



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**points of discussion**

pharmacological AMPK agonists induce RUNX2 & promote osteoblast dx/dt
AMPK KD mice display reduced bone mass

prev reports: involvement of AMPK in osteogenic differentiation of human adipose tissue-derived MSCs
first evidence identifying autophagy as a key player during AMPK-dependent osteogenic differentiation

mTOR activation is crucial for MSC osteogenic differentiation: autophagy-dependent or independent?


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thank you