

# Control of Adipogenesis by S6K1-Mediated Epigenetic Modifications



Research center  
*For*Epigenome Regulation  
선도연구센터 기초의약학분야(MRC)



Research Center for Epigenome Regulation (RCER)  
School of Pharmacy  
Sungkyunkwan University  
Jeung-Whan Han

# **Conflict of interest disclosure**

**None**

**Committee of Scientific Affairs**



**Committee of Scientific Affairs**

# **Contents**

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**Part I . Nuclear translocation of S6K1.**

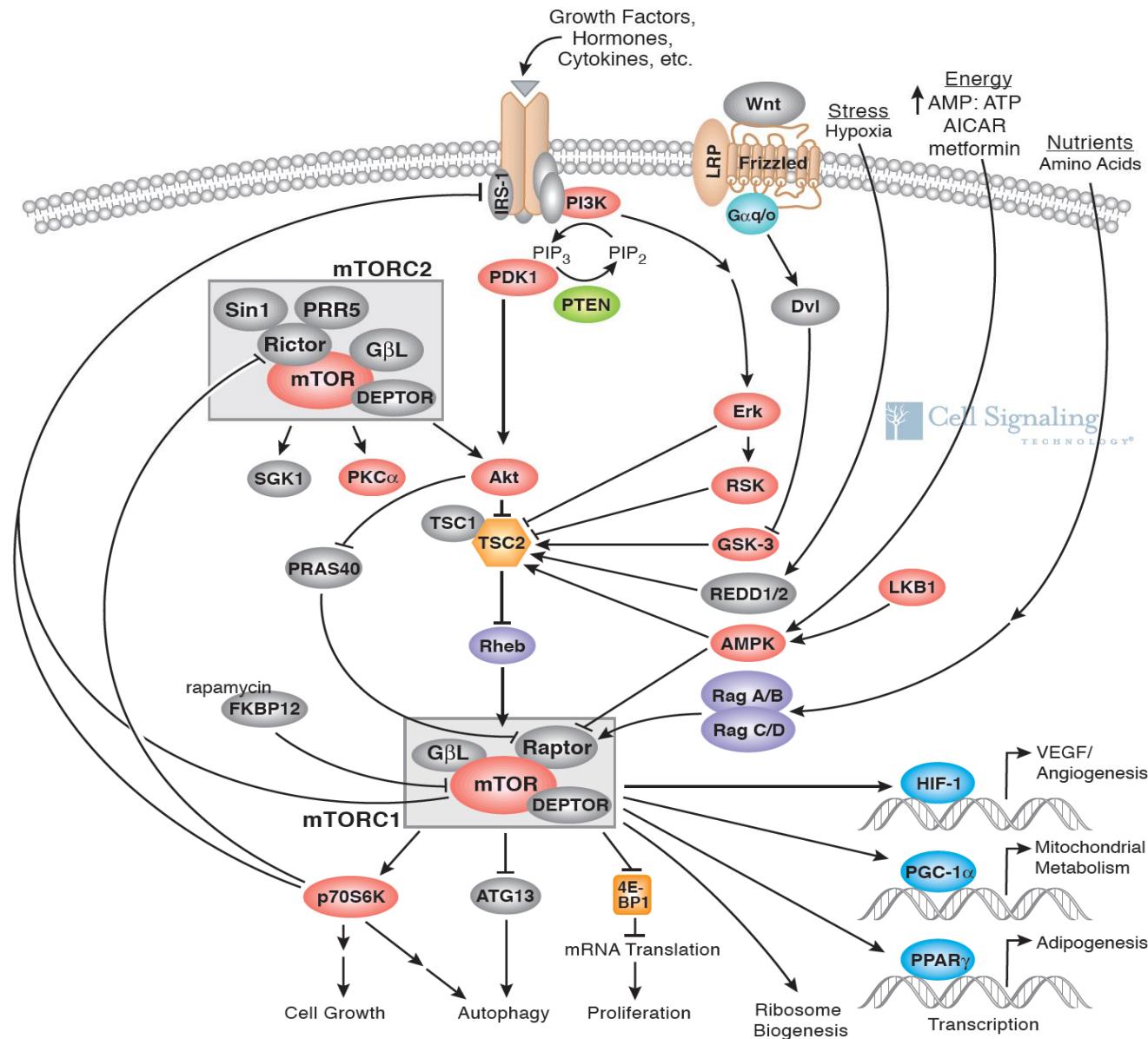
**Part II. S6K1 interacts with, and phosphorylates H2B at S36.**

**Part III. S6K1-mediated H2BS36 phosphorylation regulates  
H3K27me3.**

**Part IV. S6K1-mediated H2BS36 phosphorylation promotes  
adipogenic commitment.**

## Part I

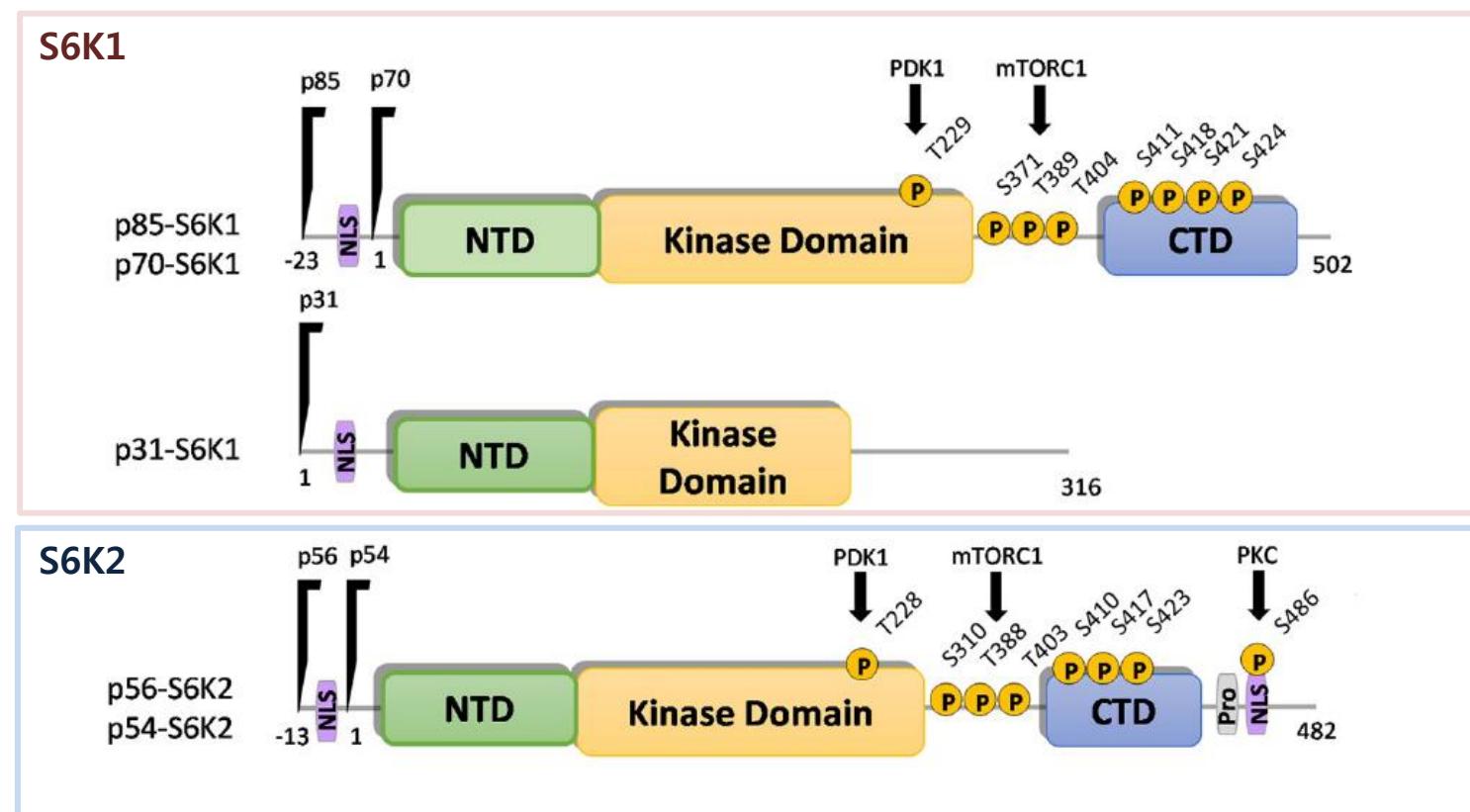
# mTOR-S6K Signaling Pathway



## Part I

# mTOR-S6K Signaling Pathway

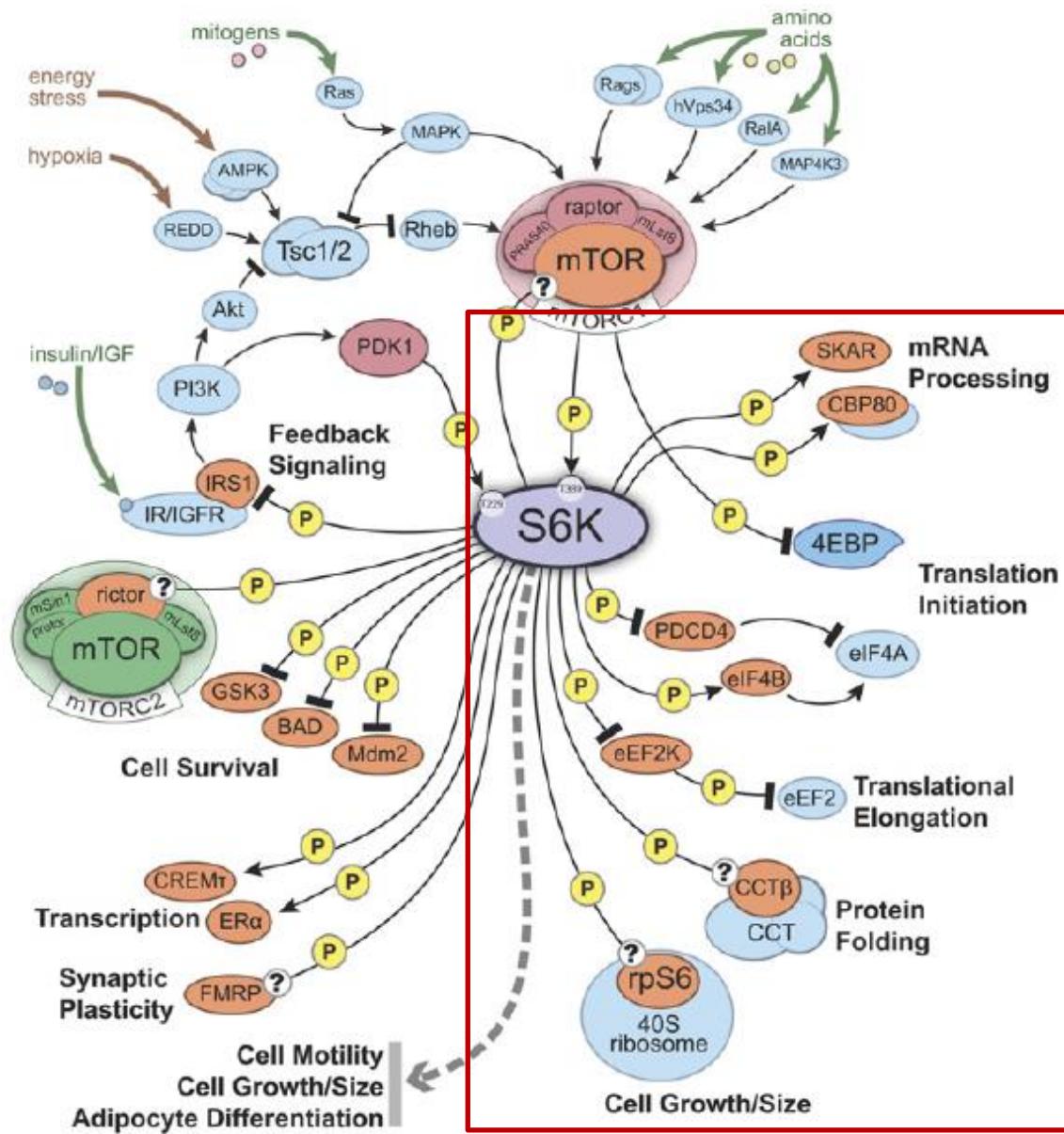
S6K1 / S6K2 isoforms : From two different genes  
P85 S6K1 / p70 S6K1 / p31 S6K1 : Alternative splicing variants



NTD: Nterminal domain; CTD: C-terminal domain; NLS: nuclear localization signal;  
Pro: proline-rich domain; P: phosphorylated residue.

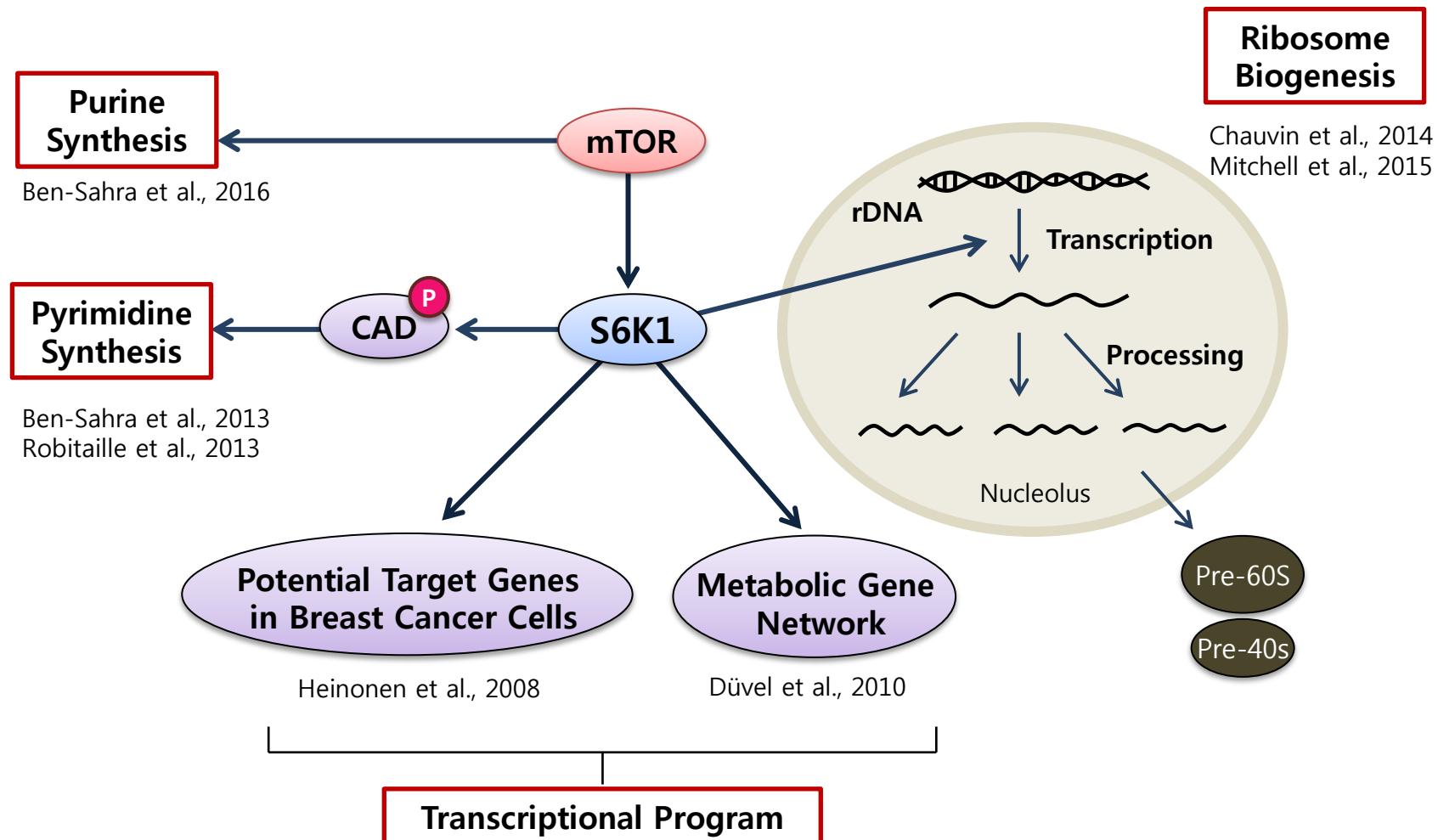
## Part I

# Substrates of S6K1 (Translational Function)



## Part I

# Substrates of S6K1 (Pre-translational Function)



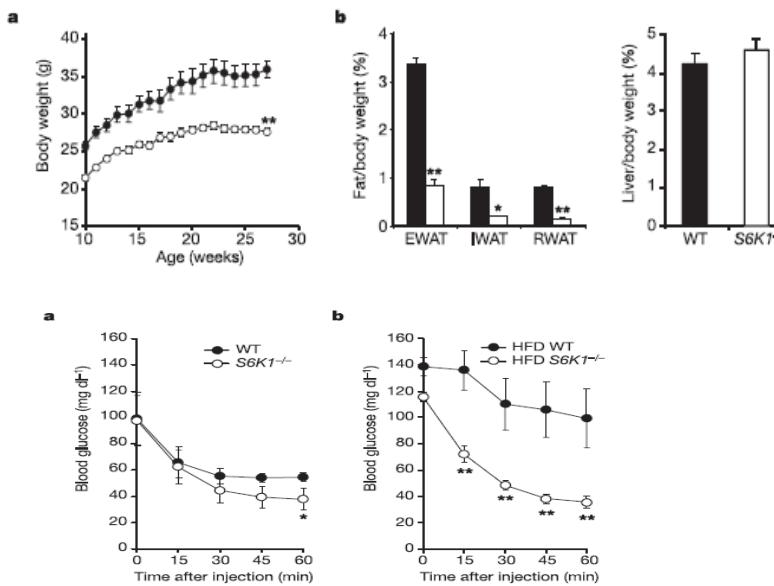
## Part I

# S6K1 in Metabolism



**letters to nature****Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity**

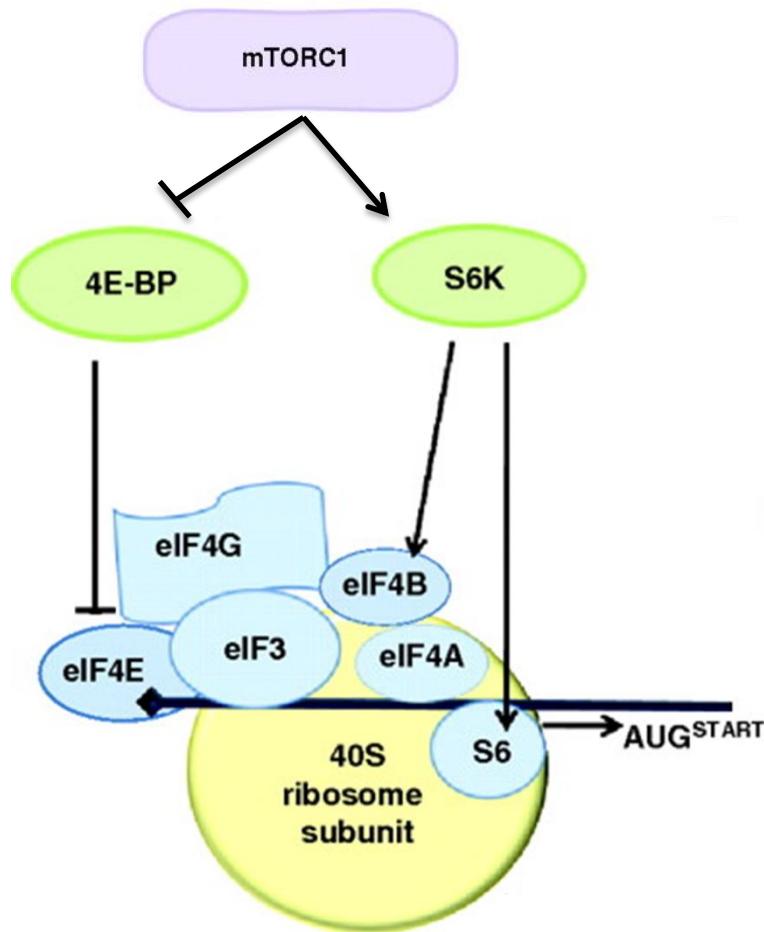
Sung Hee Um<sup>1</sup>, Francesca Frigerio<sup>1</sup>, Mitsuhiro Watanabe<sup>2</sup>, Frédéric Picard<sup>2\*</sup>, Manel Joaquin<sup>1</sup>, Melanie Sticker<sup>1</sup>, Stefano Fumagalli<sup>1</sup>, Peter R. Allegrini<sup>3</sup>, Sara C. Kozma<sup>1\*</sup>, Johan Auwerx<sup>2</sup> & George Thomas<sup>1</sup>



## Part I

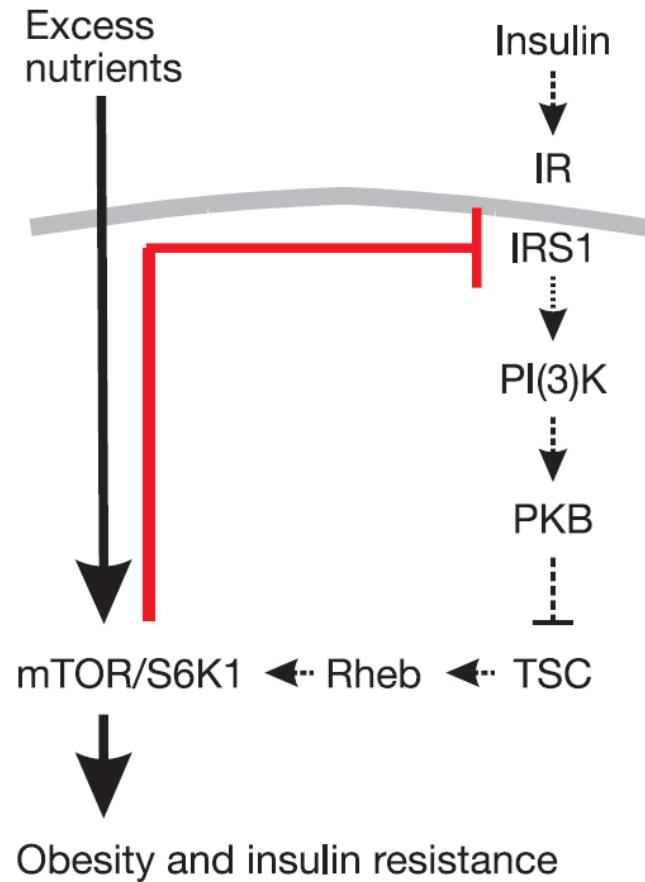
# Molecular Roles of p70 S6K in Cytoplasm

### Protein Synthesis



Russell R C. *Development* (2011)

### Phosphorylation of IRS1



Sung Hee Um. *Nature* (2004)

# Question.

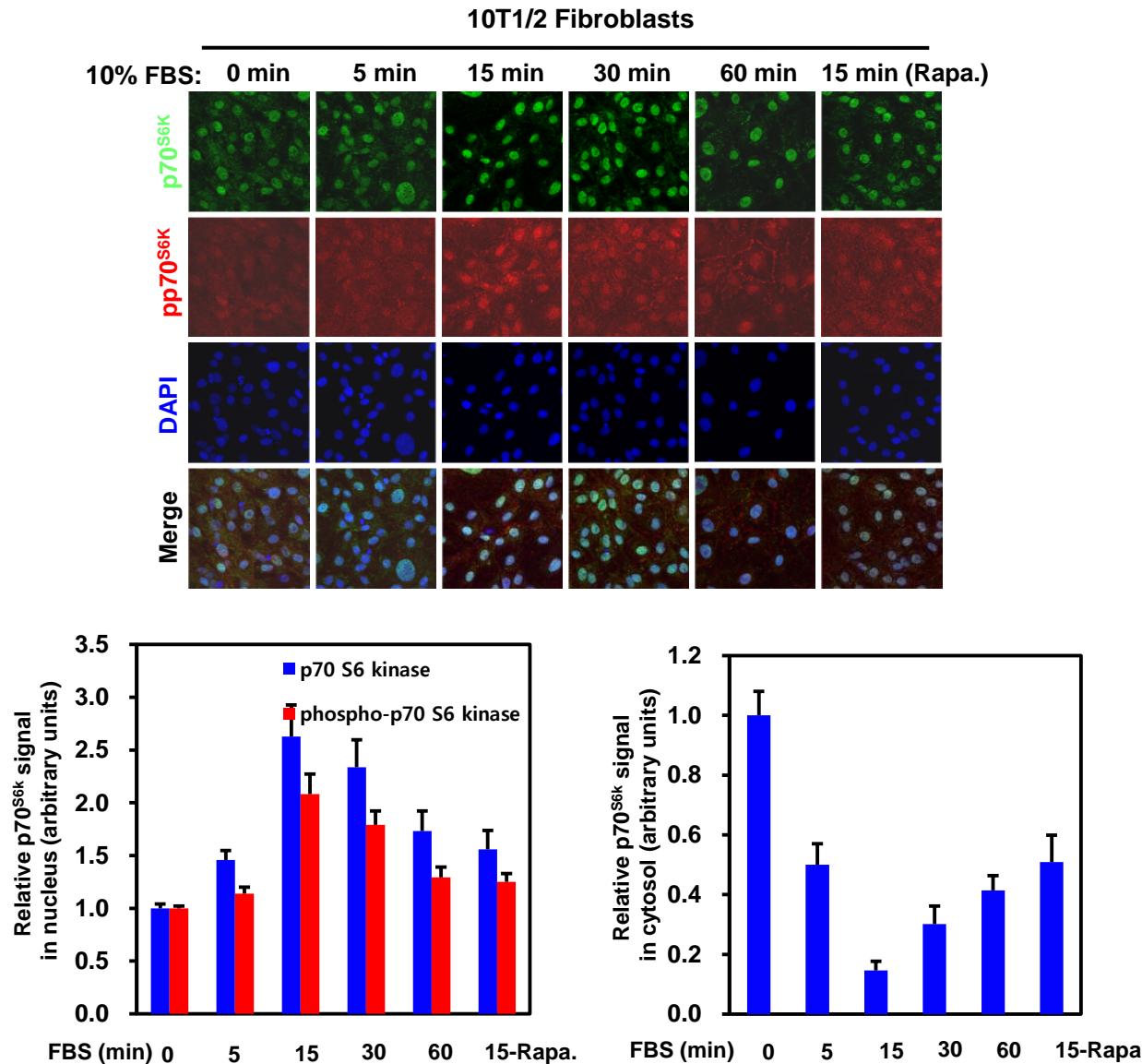
1. What is the mechanism underlying physiological functions of S6K1?

2. Can S6K1 act as a transcriptional regulator?

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## Part I

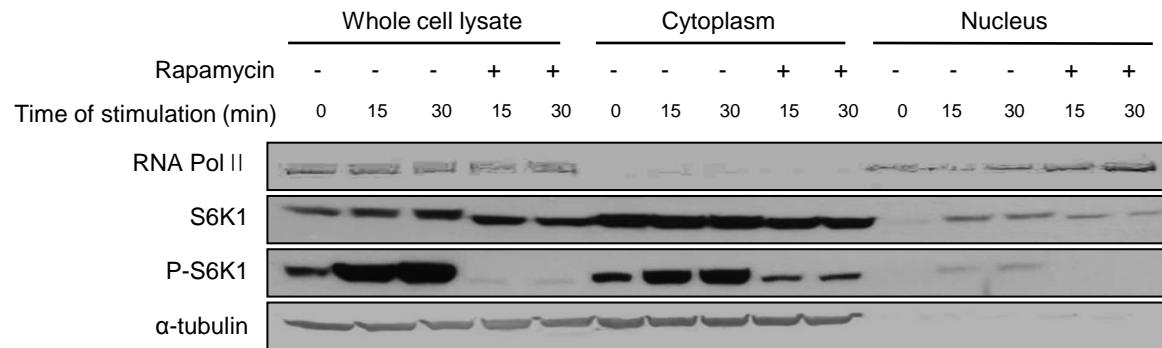
### 1. Nuclear translocation of p70 S6K1.



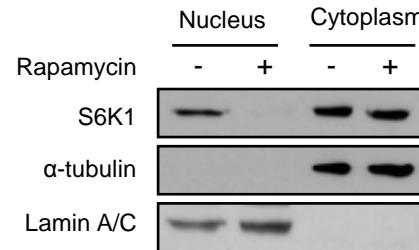
## Part I

### 1. Nuclear translocation of p70 S6K1.

HeLa

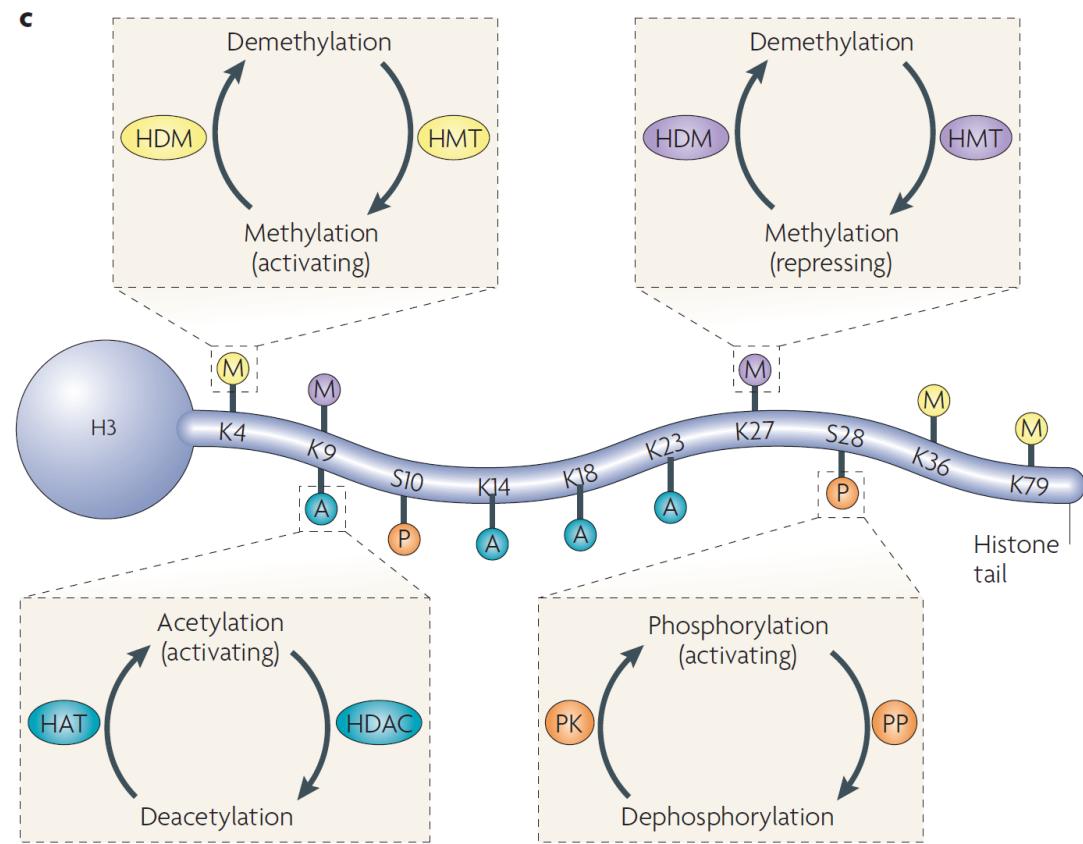
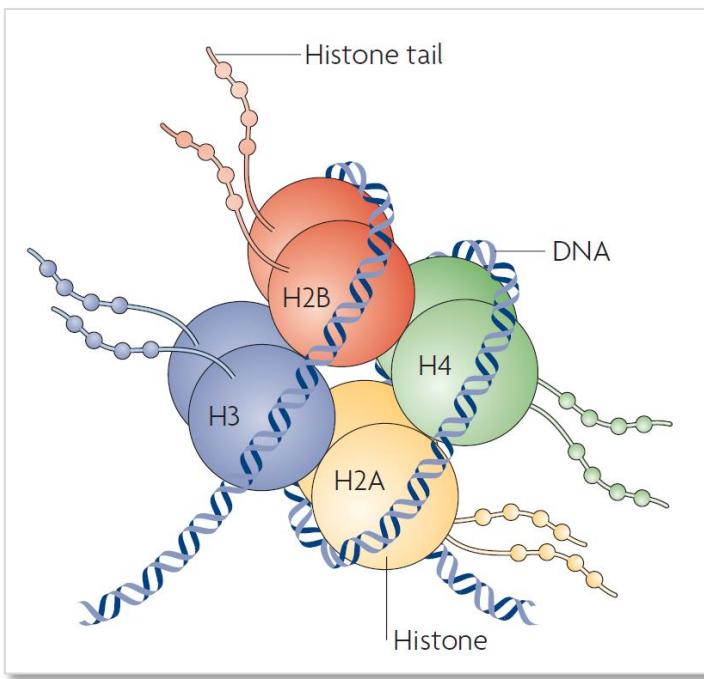


10T1/2 Fibroblast



## Part II

# Histone Post-translational Modifications

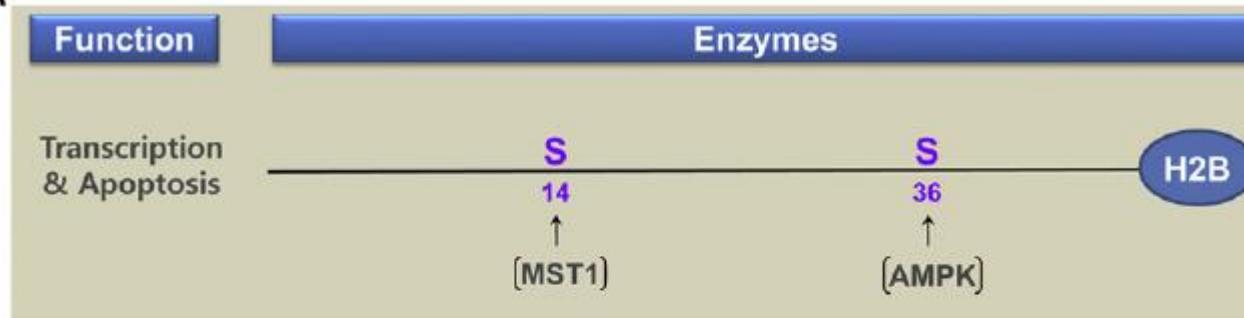


## Part II

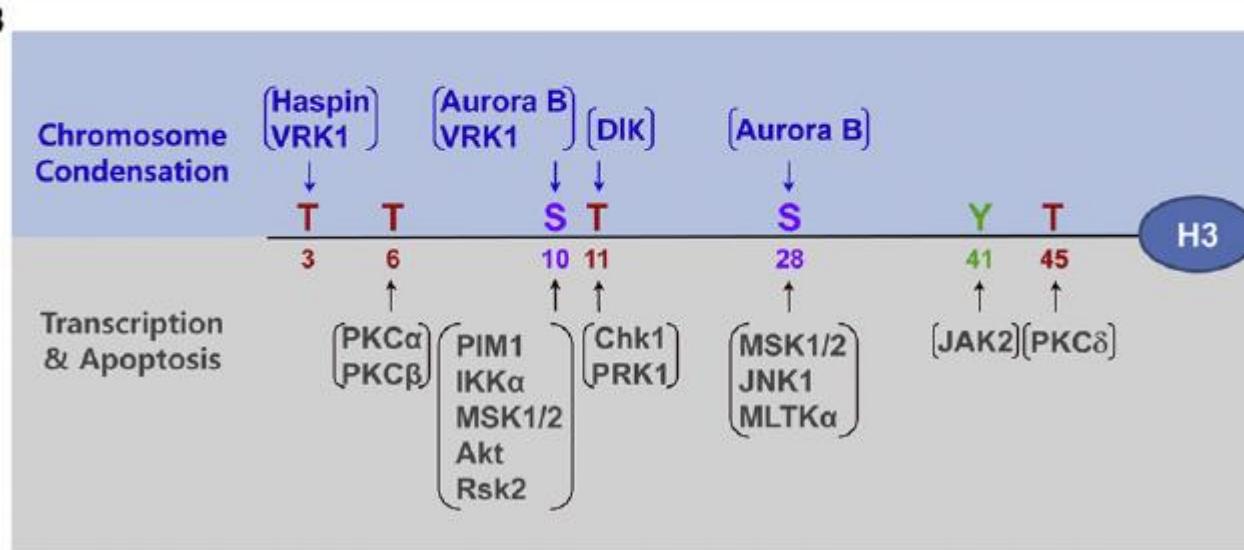
# Kinases and Histone PTMs

Fig 1. Kinases that Modify Histones H2B and H3 and Residues Modified

A



B



## Part II

# Phosphorylation Sites in Histones



## *Homo sapiens*

CENTERFO RBIOLIGI CALSEQU ENCEANA LYSIS CBS		NetPhos 2.0 Server - prediction results					
Technical University of Denmark							
126 Sequence							
MPEPAKSAPAKKGSKKAVTKAQKKDGKKRKRKESYSIVVYVVLQVHPDTGISSKAMGMNSFVNDFERIAGEASR							
LAHYMKRSTITSREIQTAVRLLLPGEELAKHAYSEGTKAVTKYTSSK				80			
.....S.....S.....S..SYS.Y.....S.....S.....S.				160			
.....T.TS.....T.....S.T.....Y.S..				160			
Phosphorylation sites predicted:							
		Ser: 9	Thr: 4	Tyr: 3			
Serine predictions							
Name	Pos	Context	Score	Pred			
Sequence	7	EPAKSAPAP	0.789	*S*			
Sequence	15	PKKGSKKAV	0.879	*S*			
Sequence	33	KAKRKRKES	0.998	*S*			
Sequence	37	SKESYSIY	0.993	*S*			
Sequence	39	KESYSIYYY	0.943	*S*			
Sequence	56	DTGISSKAM	0.717	*S*			
Sequence	57	TGISSKAMG	0.107	.			
Sequence	65	GIMNSFVNND	0.012	.			
Sequence	79	AGEASRLAH	0.084	.			
Sequence	88	YNKRSTITS	0.229	.			
Sequence	92	STITSREIQ	0.924	*S*			
Sequence	113	KHAVSEGTK	0.640	*S*			
Sequence	124	TKYTSSK--	0.924	*S*			
Sequence	125	KYTSSK---	0.057	.			

*Mus musculus*

<b>CENTERFOR BIOLOGI CALSEQU ENCEANA LYSIS CBS</b>	<h1>NetPhos 2.0 Server - prediction results</h1> <h2>Technical University of Denmark</h2>									
<hr/>										
126 Sequence										
MPELAKSAPAKKGSKKAVTKAQKKDGKKRKRKRSRKESVSIYVVKVLQVHPDTGISKAMGI	MNSFVN	DIFERIA	NEASR	80						
LAHYNKRSTITSREIQTSVRLLLPGELAKHVASEGTKAVTKYTSK				160						
.....S.....S.....S..SYS.Y.....S.....S.....				80						
.....T.TS.....TS.....S.T.....V.S..				160						
Phosphorylation sites predicted:			Ser: 10	Thr: 4	Tyr: 3					
<hr/>										
Serine predictions										
Name	Pos	Context	Score	Pred						
		v								
Sequence	7	ELAKSAPAP	0.666	*S*						
Sequence	15	PKKGSKKAV	0.879	*S*						
Sequence	33	KAKRSRKES	0.998	*S*						
Sequence	37	SRKESVSVIV	0.993	*S*						
Sequence	39	KESVSTYWW	0.943	*S*						
Sequence	56	DTGISSKAM	0.717	*S*						
Sequence	57	TGISSKAMG	0.107	.						
Sequence	65	GIMNSFVN	0.012	.						
Sequence	79	ANEASRPLAH	0.030	.						
Sequence	88	VNKRASTITS	0.229	.						
Sequence	92	STITSREIQ	0.924	*S*						
Sequence	98	E1QTSVALL	0.829	*S*						
Sequence	113	KHAVSEGTK	0.640	*S*						
Sequence	124	TKVTSK---	0.887	*S*						
		^								
<hr/>										

H2B

# KKRKRSRKESY

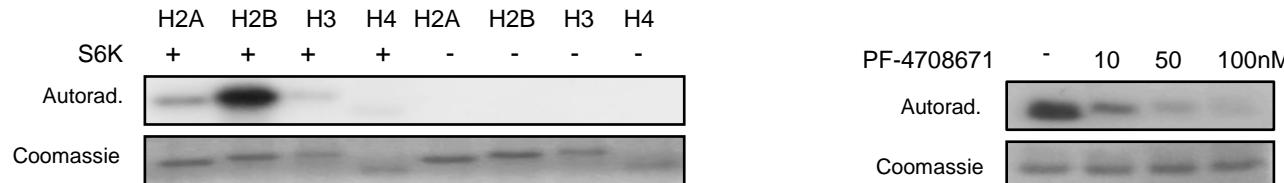
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Sequence: <http://www.uniprot.org/uniprot/Q64524>

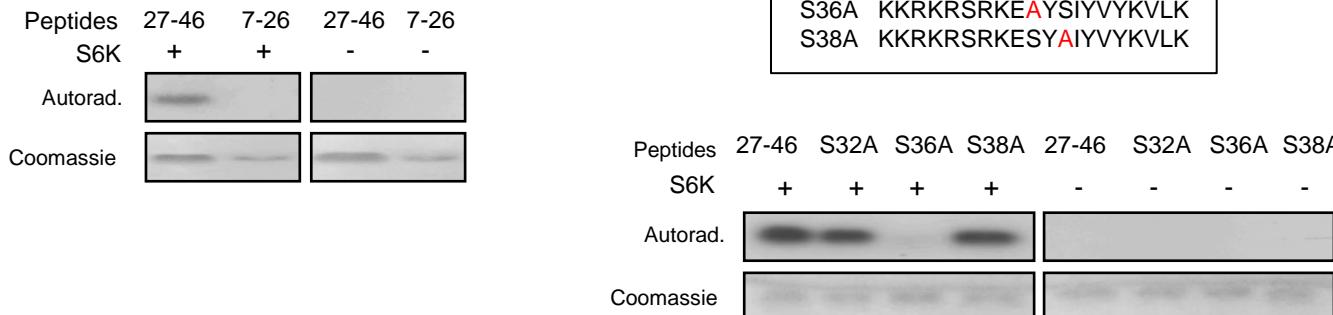
## Part II

### 2. S6K1 phosphorylates H2B in vitro.

#### Recombinant Histones



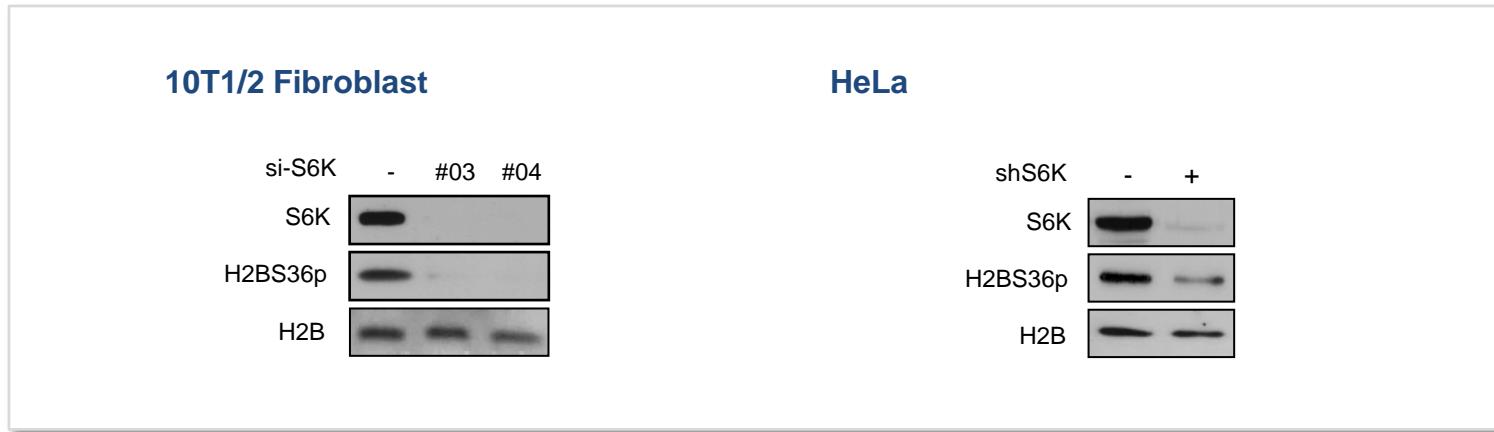
#### H2B peptides



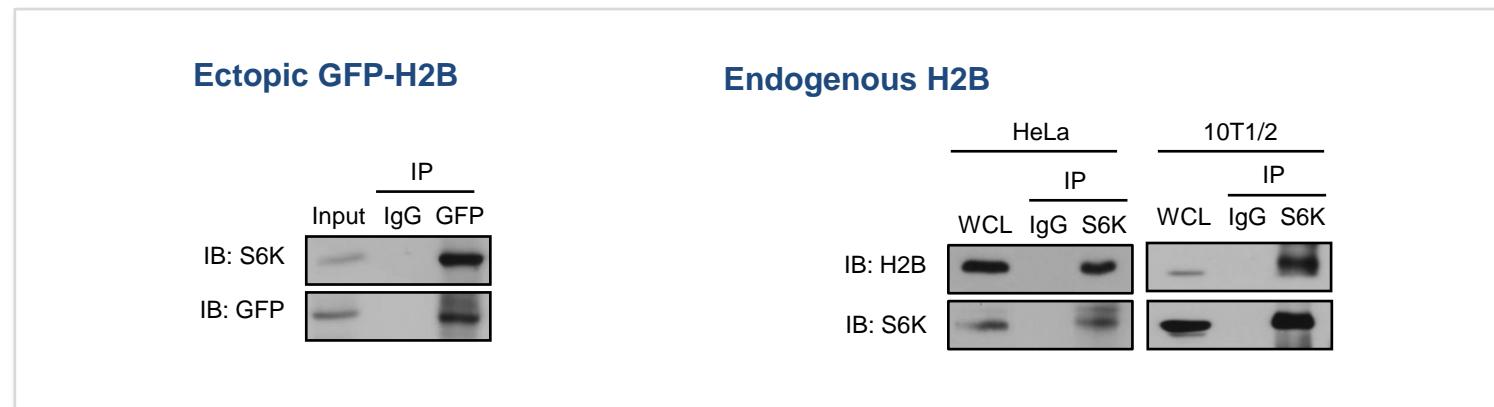
## Part II

### 3. S6K1 interacts with, and phosphorylates H2B in vivo.

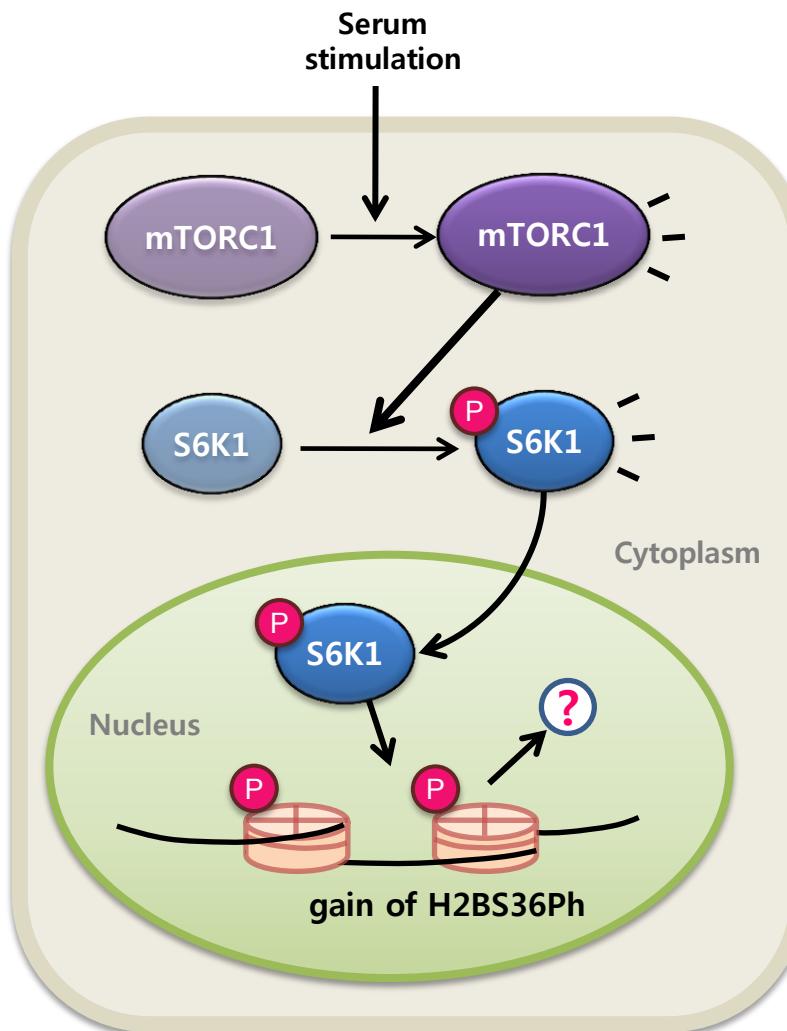
#### Western blot



#### Immunoprecipitation



# Summary 1



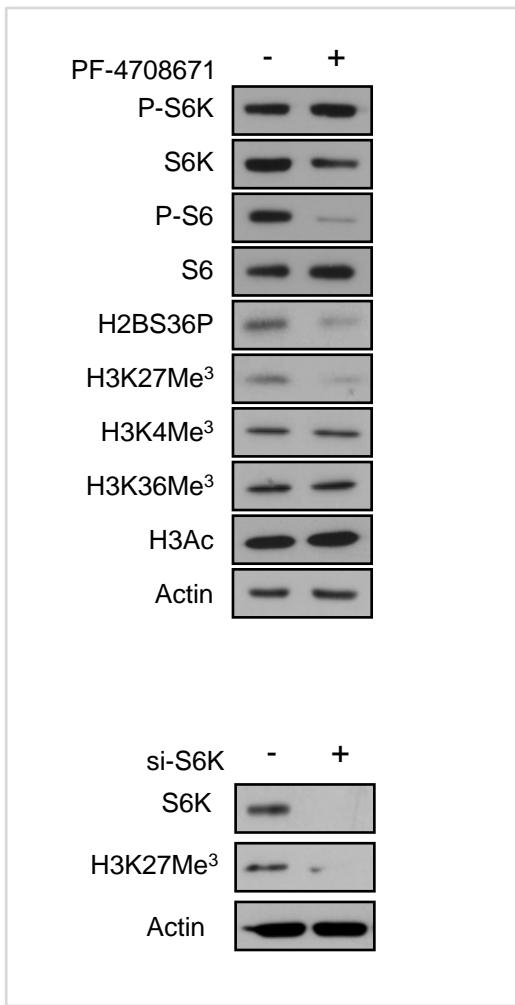
Nuclear S6K1 directly interacts with, and phosphorylates H2B at S36.

Question:  
Does S6K1 affect other histone modifications?

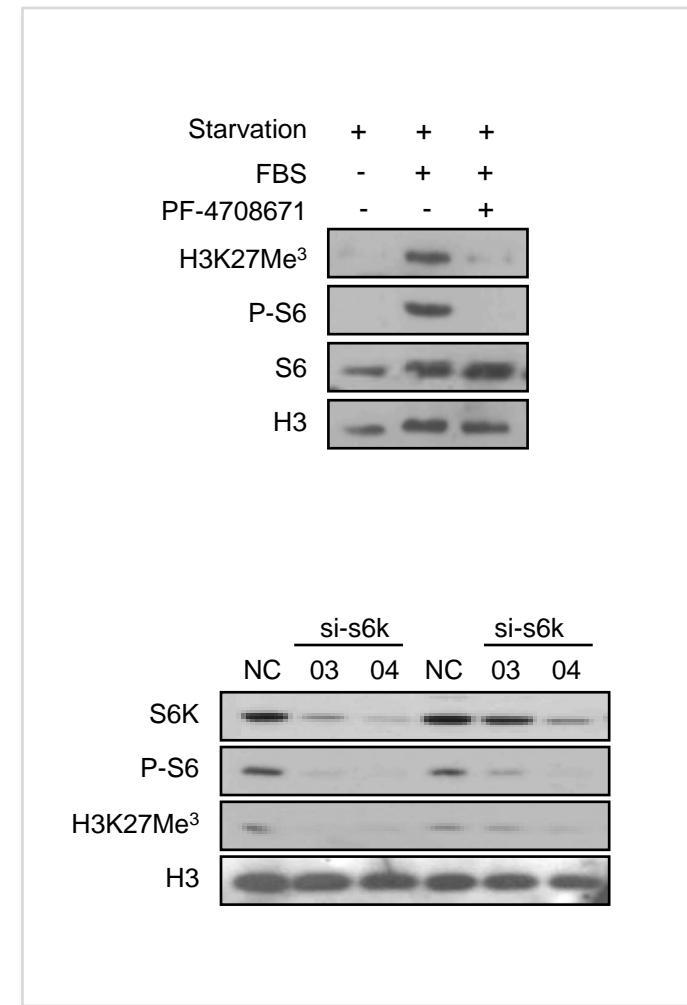
### Part III

## 4. S6K1 is required for H3K27 trimethylation.

10T1/2



HeLa

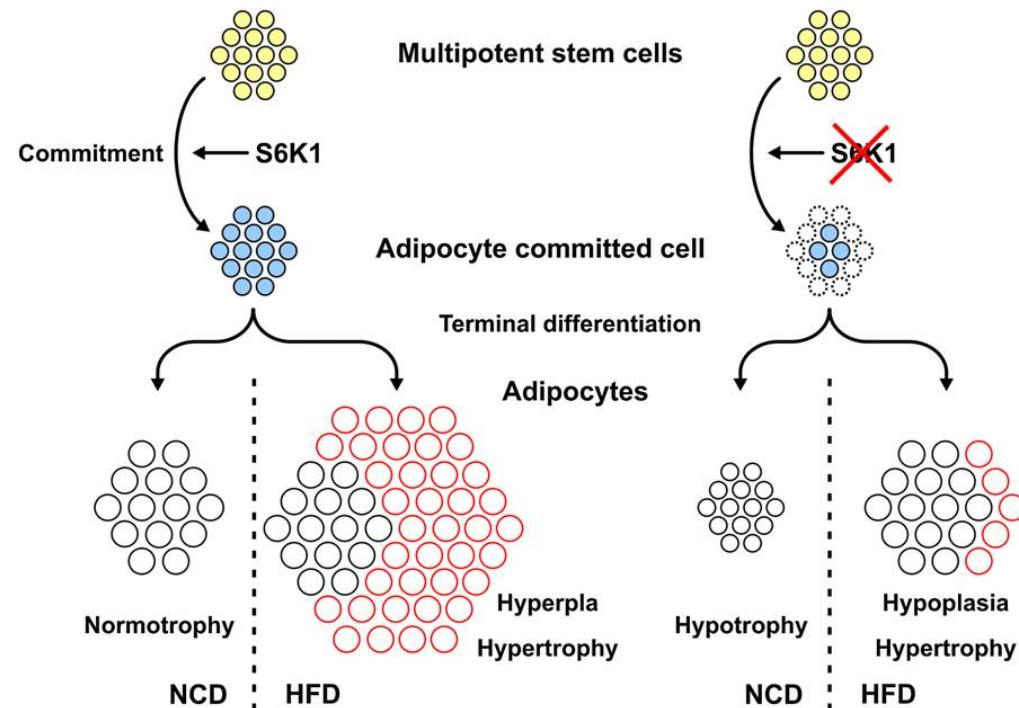
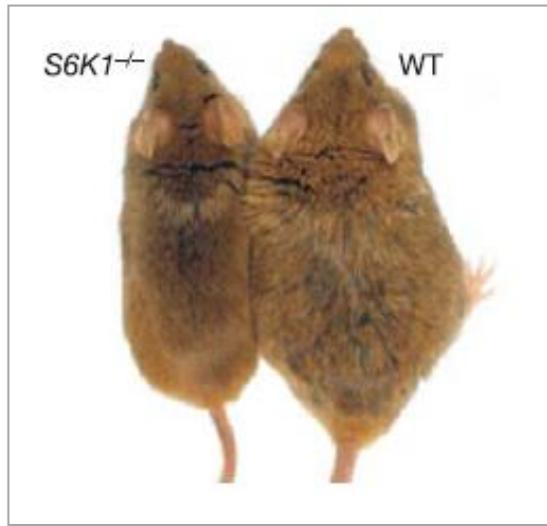


# S6K1 in Metabolism



## S6K1 Plays a Critical Role in Early Adipocyte Differentiation

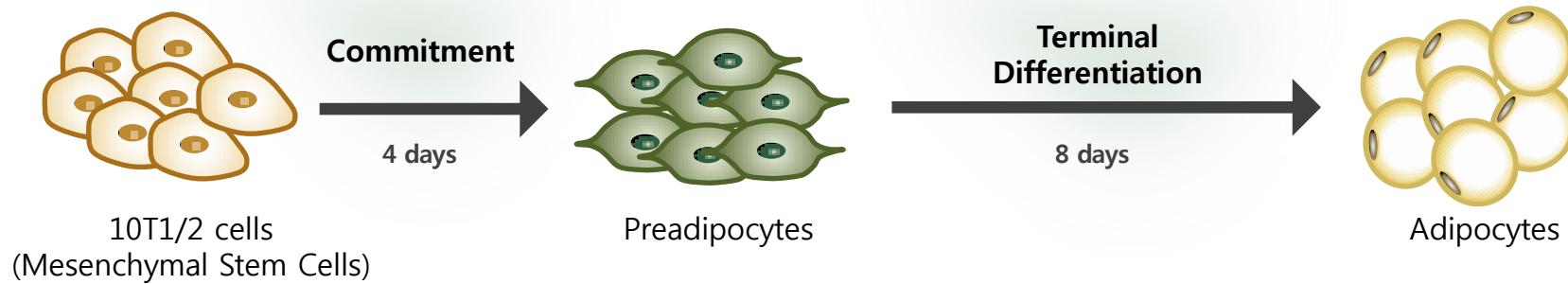
Larissa S. Camevalli,<sup>1</sup> Kouhei Masuda,<sup>1</sup> Francesca Frigerio,<sup>2,4</sup> Olivier Le Bacquer,<sup>3,5</sup> Sung Hee Um,<sup>1,6</sup> Valentina Gandin,<sup>3</sup> Ivan Topisirovic,<sup>3</sup> Nahum Sonenberg,<sup>3</sup> George Thomas,<sup>1</sup> and Sara C. Kozma<sup>1,\*</sup>



### Part III

## Adipogenesis from Mesenchymal Stem Cell line

day-4	day0	day1	day2	day3	day4	day5	day6	day7	day8
BMP4 MSCs	MDI Predipocytes		Insulin		Insulin		Insulin		Adipocytes



**M** (IBMX)

Increases intracellular cAMP level to activate protein kinase A

**D** (Dexamethasone)

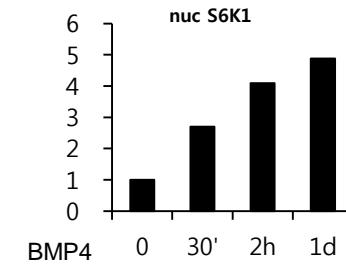
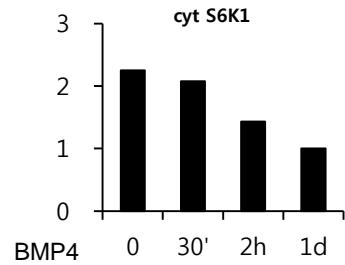
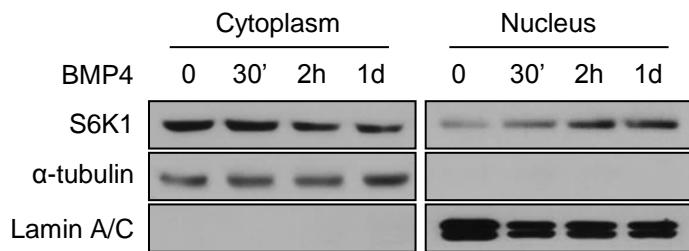
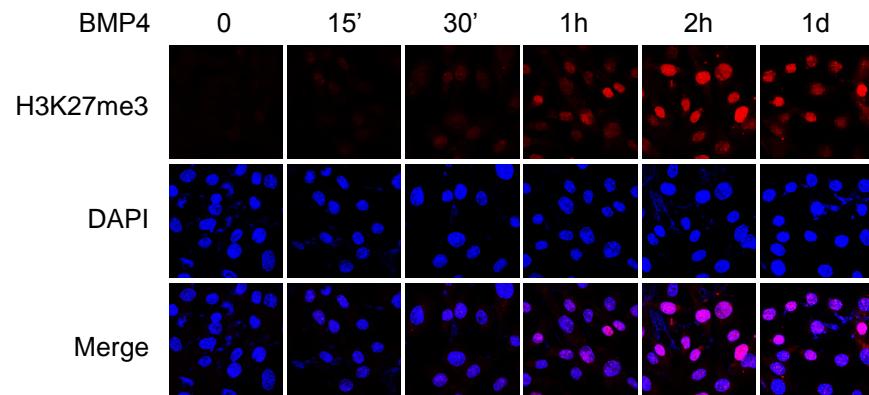
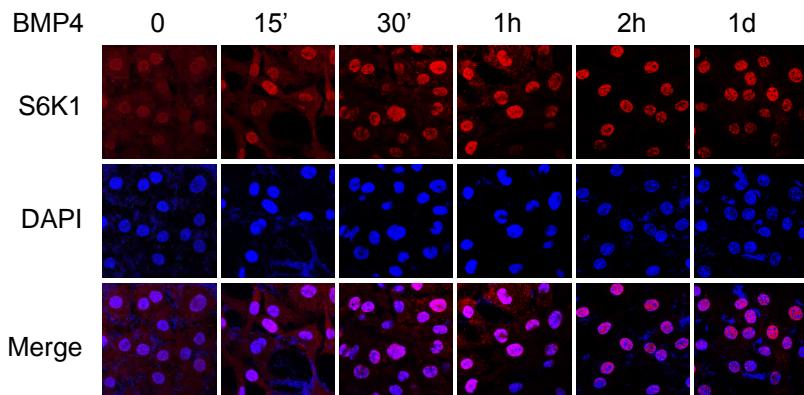
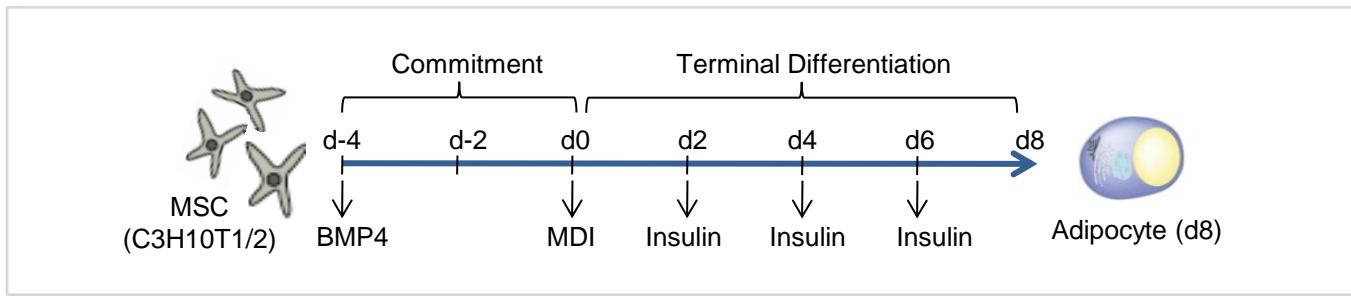
Binds and activates glucocorticoid receptor (GR)

**I** (Insulin)

Stimulates insulin like growth factor 1 (IGF1) pathway

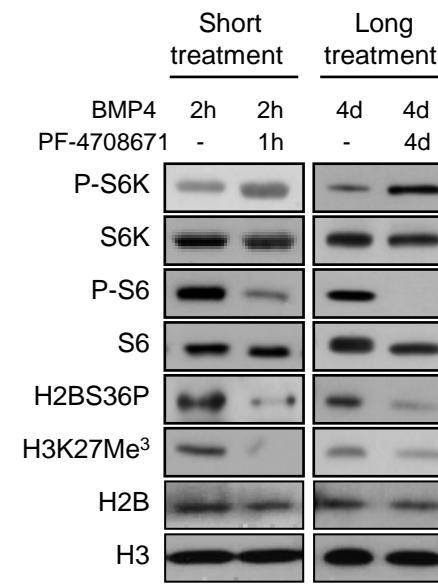
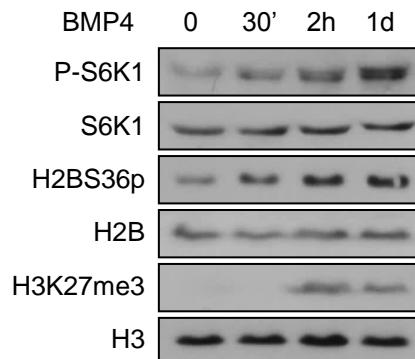
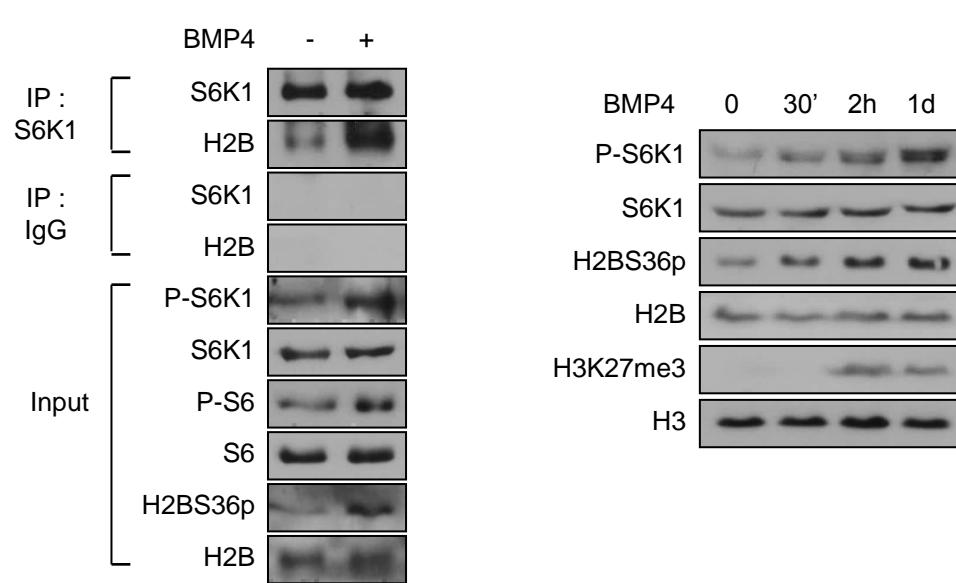
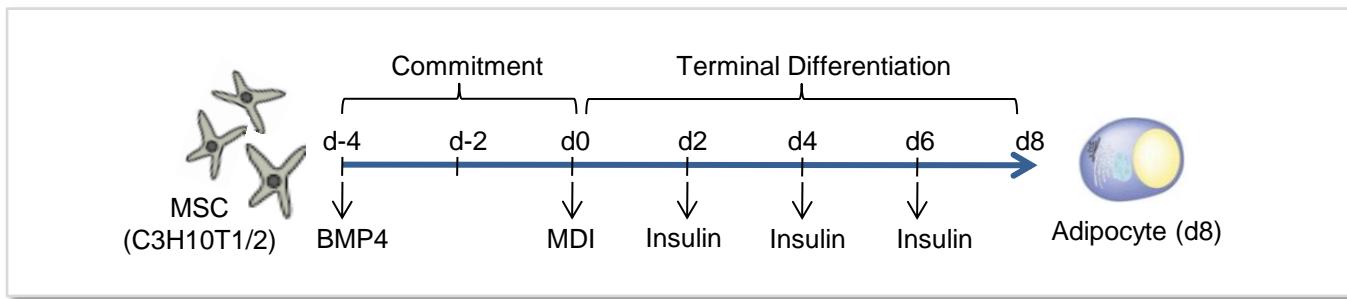
### Part III

## 4. S6K1 is required for H3K27 trimethylation.

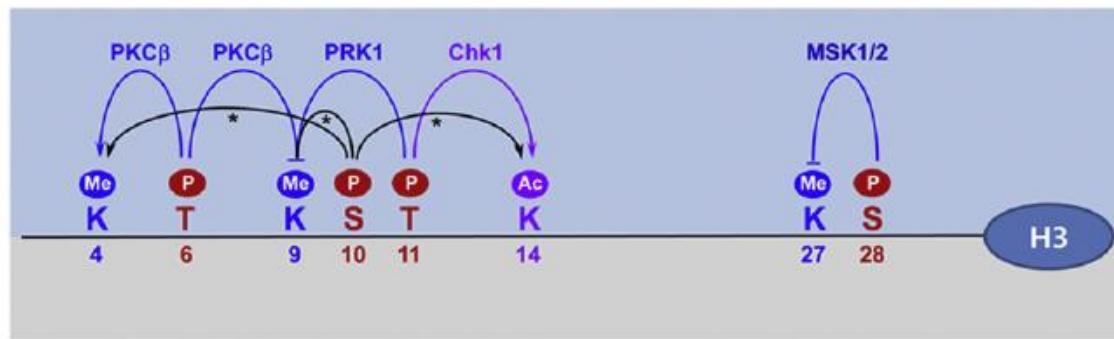


### Part III

## 4. S6K1 is required for H3K27 trimethylation.



# Histone Phosphorylation and Crosstalk

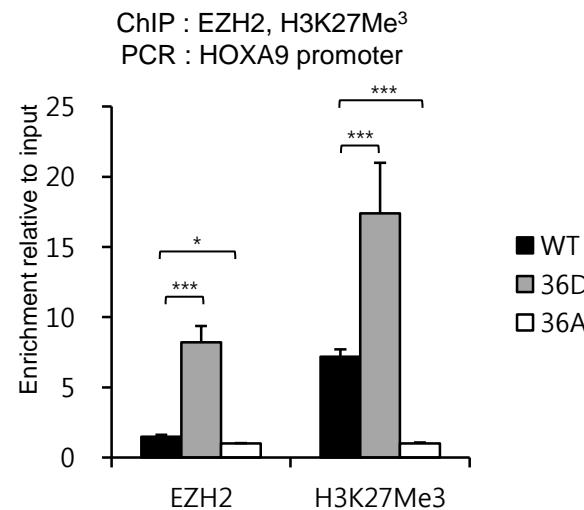
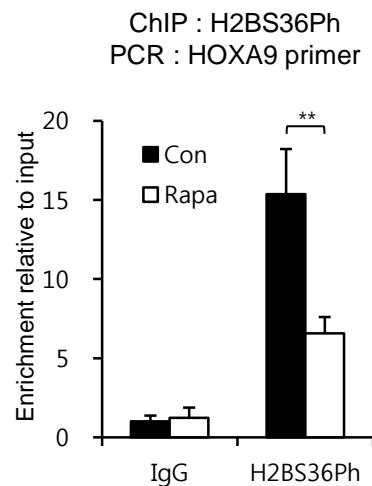
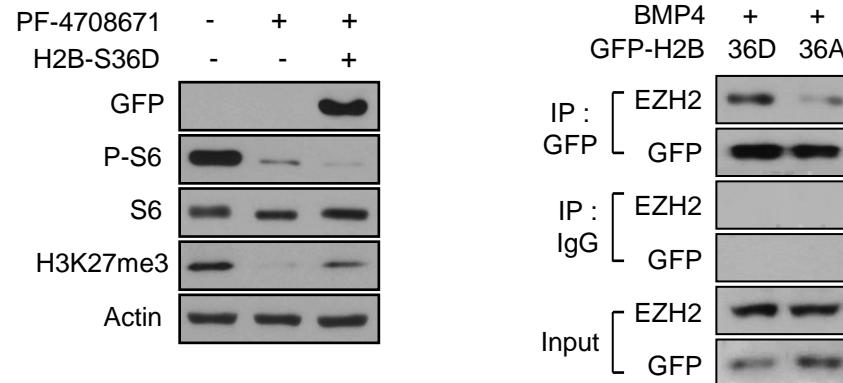


Crosstalk between Histone Phosphorylation and Other Modifications

## Part III

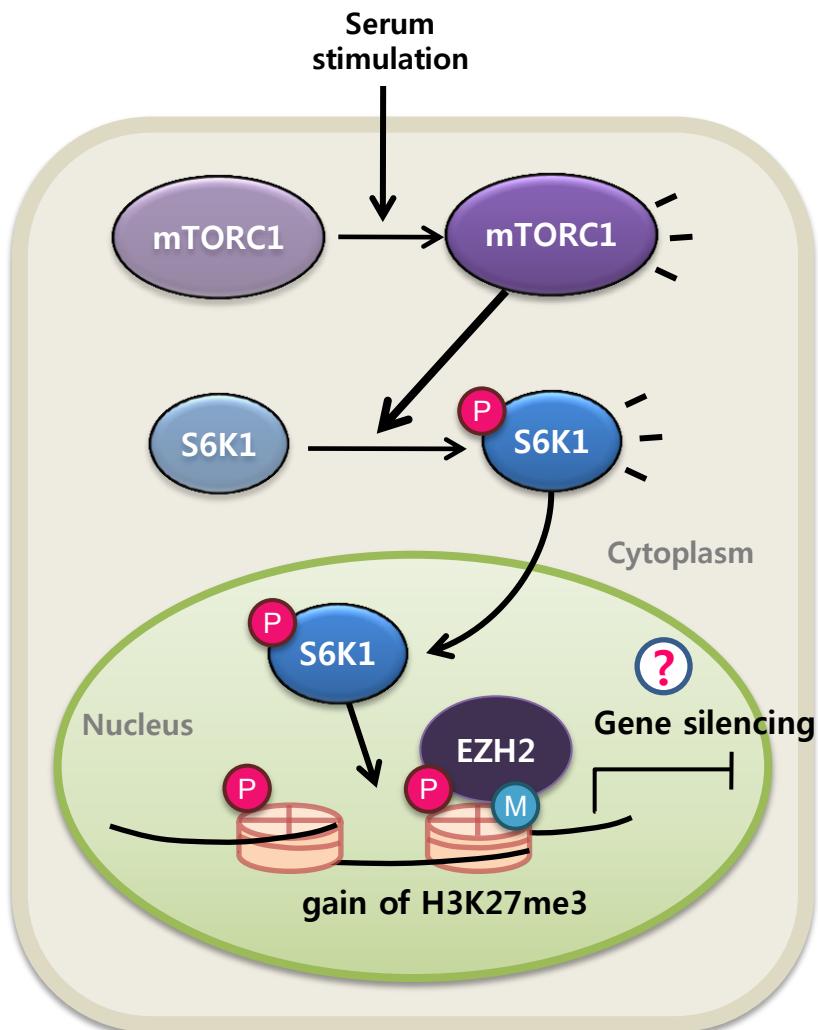
### 7. S6K1-mediated H2BS36Ph is required for EZH2 effects.

H2B-WT : Normal H2B  
 H2B-S36D : Phospho-mimic  
 H2B-S36A : Phospho-resistance



## Part III

### Summary 2



S6K1-mediated H2Bp phosphorylation promotes H3K27me3 during adipogenic commitment.

What genes are suppressed by S6K1-H2Bp-H3K27me3 axis during adipogenic commitment?

# Epigenetic Regulation of Adipogenesis

Review

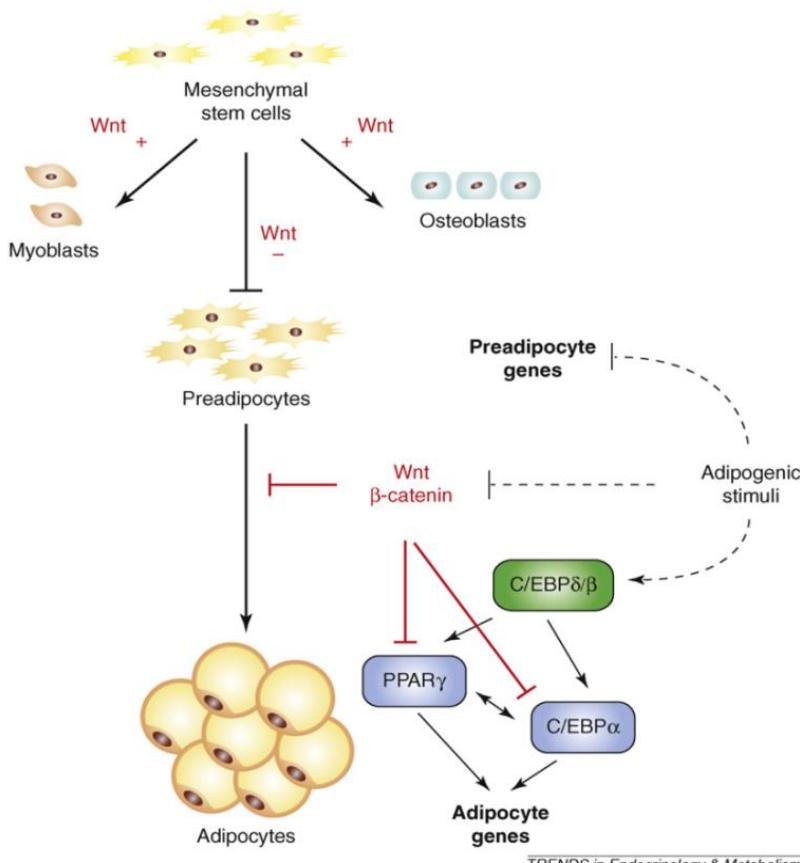
Cell  
PRESS

## Adipogenesis and WNT signalling

Constantinos Christodoulides<sup>1,2</sup>, Claire Lagathu<sup>1</sup>, Jaswinder K. Sethi<sup>1</sup> and Antonio Vidal-Puig<sup>1</sup>

<sup>1</sup> Institute of Metabolic Science, MRC Centre for Obesity and Associated Diseases, Biochemistry, University of Cambridge, Addenbrooke's Hospital, Cambridge, CB2 0QQ, UK

<sup>2</sup> Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, OX3 7LJ, UK



## Histone H3K27 methyltransferase Ezh2 represses Wnt genes to facilitate adipogenesis

Lifeng Wang<sup>a</sup>, Qihuang Jin<sup>a</sup>, Ji-Eun Lee<sup>a</sup>, I-hsin Su<sup>b,1</sup>, and Kai Ge<sup>a,2</sup>

<sup>a</sup>Nuclear Receptor Biology Section, Clinical Endocrinology Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892; and <sup>b</sup>Laboratory of Lymphocyte Signaling, The Rockefeller University, New York, NY 10065

Edited by Mark T. Groudine, Fred Hutchinson Cancer Research Center, Seattle, WA, and approved March 12, 2010 (received for review January 4, 2010)

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Original Full Length Article

**Wnt6, Wnt10a and Wnt10b inhibit adipogenesis and stimulate osteoblastogenesis through a β-catenin-dependent mechanism**

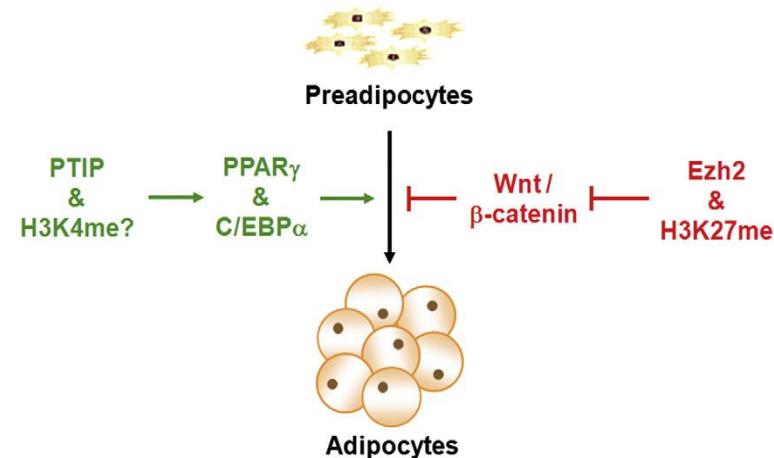
William P. Cawthon<sup>a</sup>, Adam J. Bree<sup>a</sup>, Yao Yao<sup>a</sup>, Baowen Du<sup>a,d</sup>, Nahid Hemati<sup>a</sup>, Gabriel Martínez-Santibáñez<sup>c</sup>, Ormond A. MacDougald<sup>a,b,\*</sup>

<sup>a</sup> Department of Molecular & Integrative Physiology, University of Michigan Medical School, Ann Arbor, MI 48105, USA

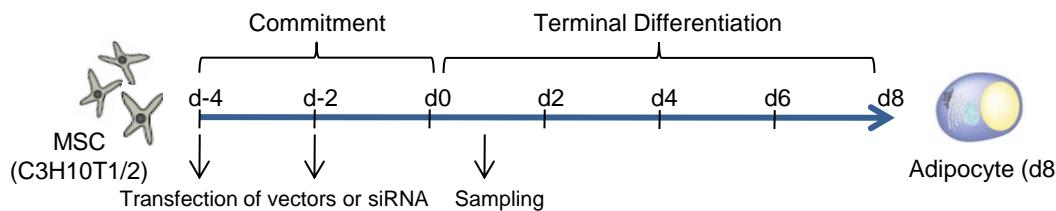
<sup>b</sup> Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI 48105, USA

<sup>c</sup> Department of Cell and Molecular Biology Program, University of Michigan Medical School, Ann Arbor, MI 48105, USA

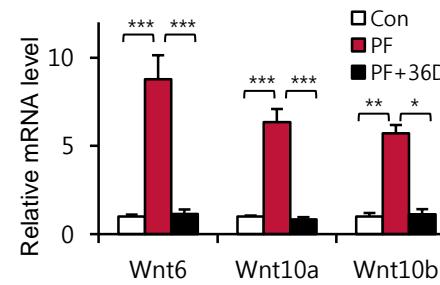
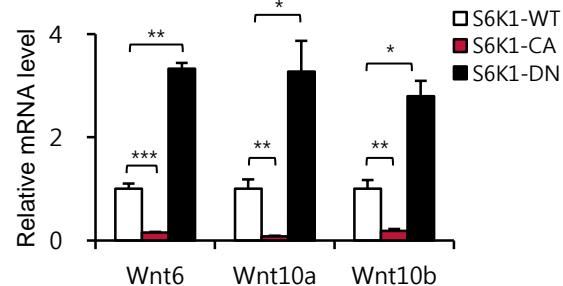
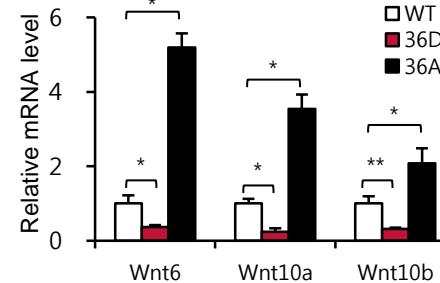
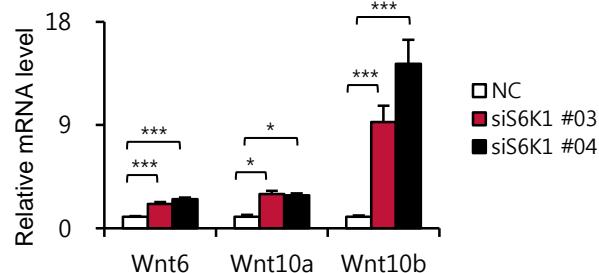
<sup>d</sup> College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, 712100, China



## 8. S6K1-mediated H2BS36Ph suppresses Wnt genes expression.



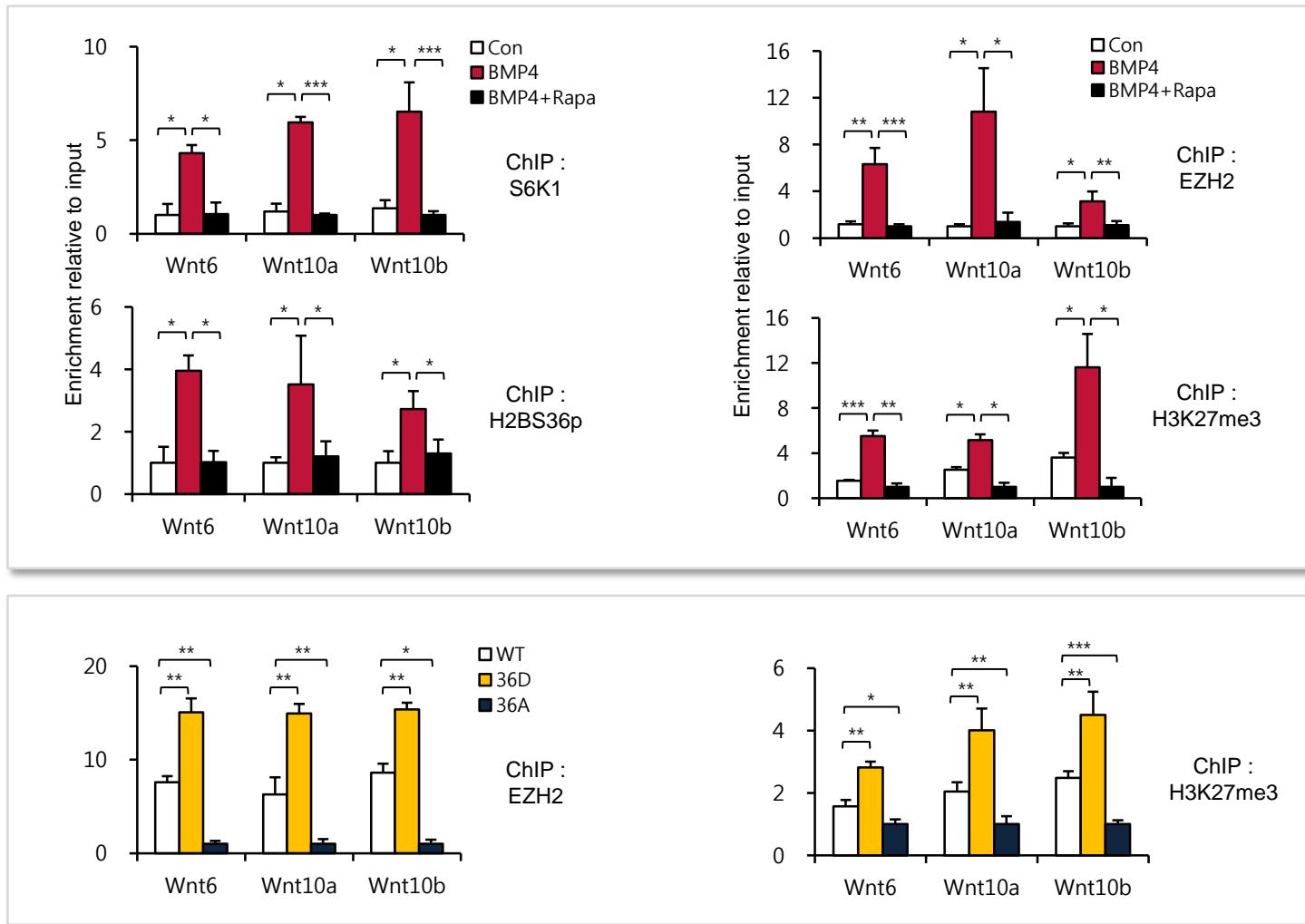
### RT-qPCR



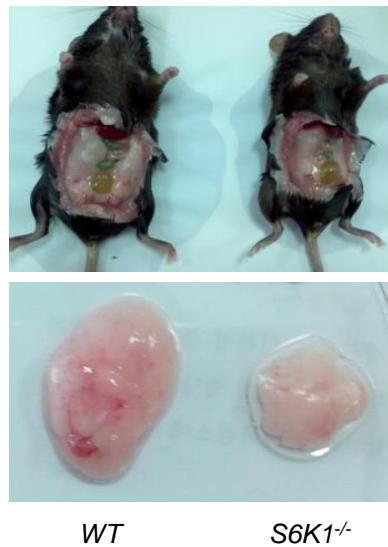
## Part IV

# 8. S6K1-mediated H2BS36Ph suppresses Wnt genes expression.

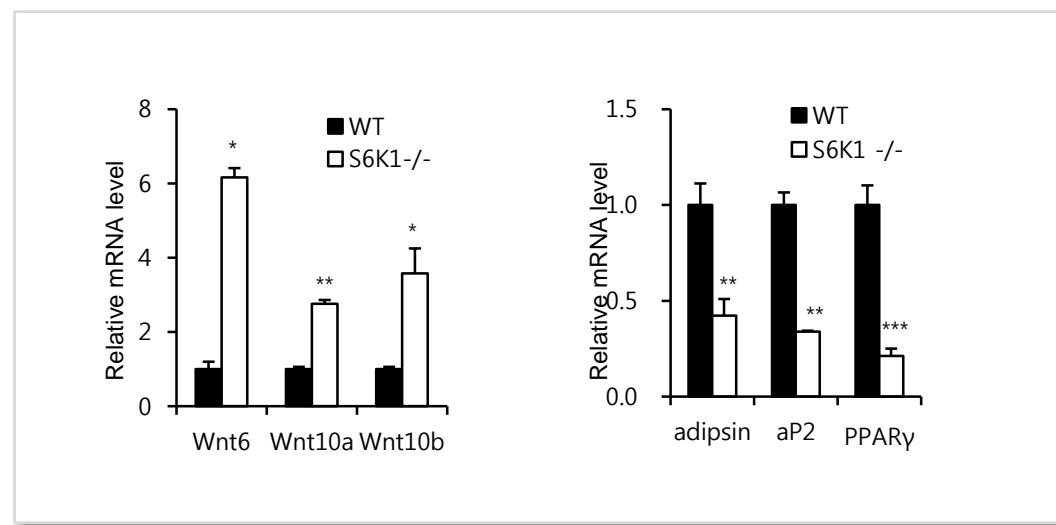
### ChIP



# 11. Expression of adipogenic genes in *S6K1* knock-out mice.

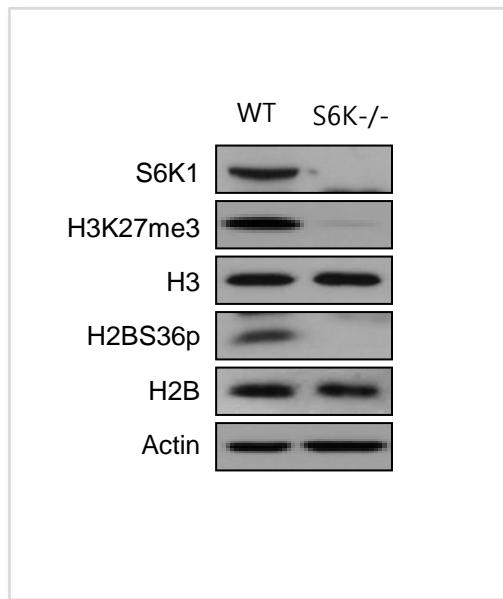


Mouse Epididymal WAT (eWAT)

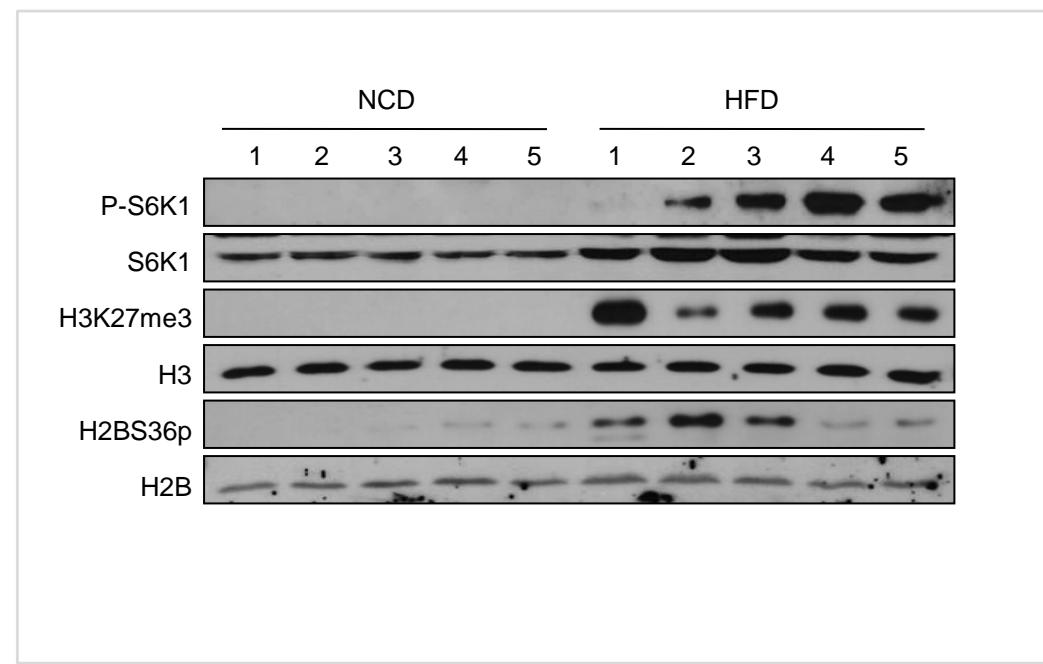


## 12. Histone modifications in S6K-deleted mice

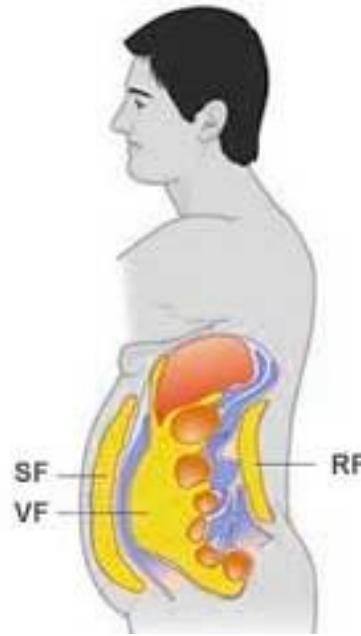
S6K K.O. mouse



Obesity-induced mice



# 13. P-S6K & histone modifications in human WAT



SF - subcutaneous (superficial) belly fat  
 VF - visceral (deep) belly fat  
 RF - retroperitoneal (back) fat

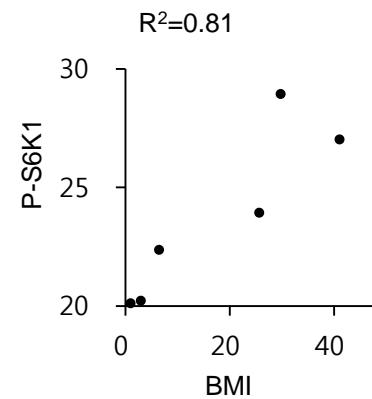
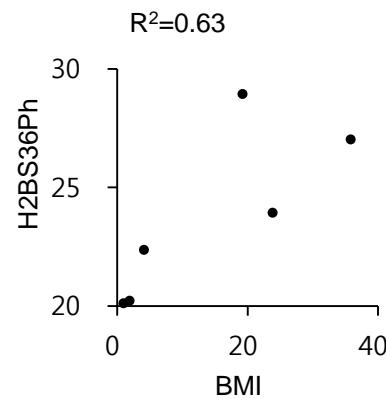
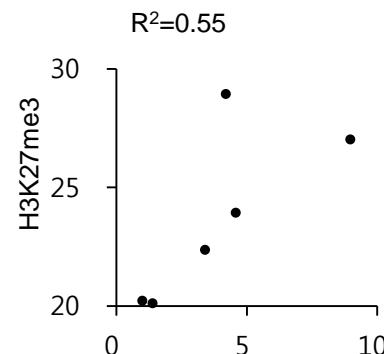
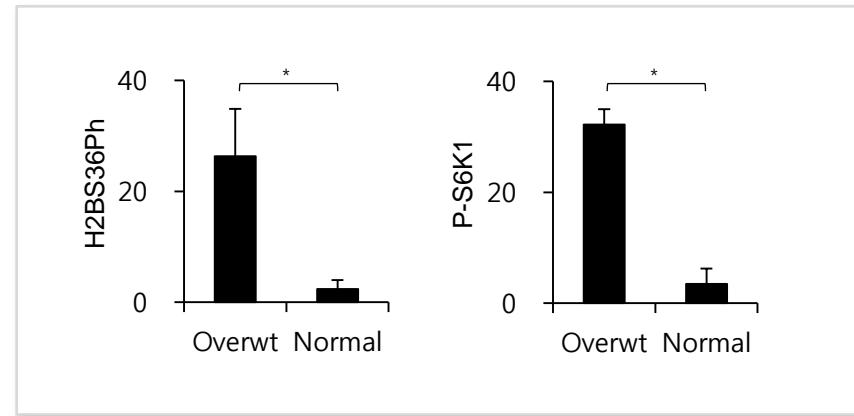
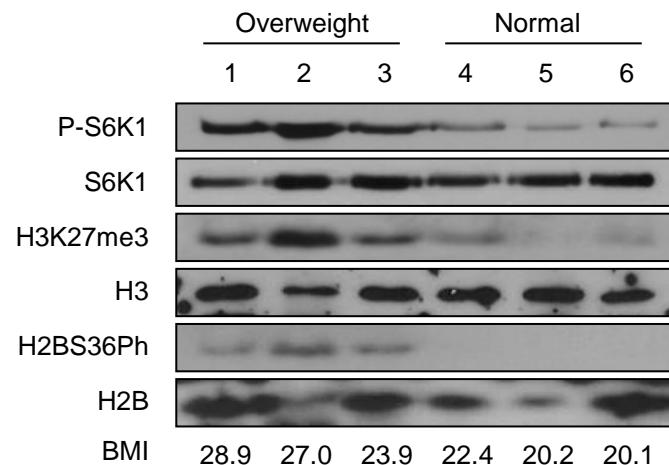
$$BMI \text{ (body mass index)} = \frac{\text{(Weight)}}{\text{(Height)}^2}$$

Underweight	< 18.5
Normal	18.5 ~ 22.9
Overweight (At Risk)	23.0 ~ 24.9
Overweight (Obese)	≥ 25.0

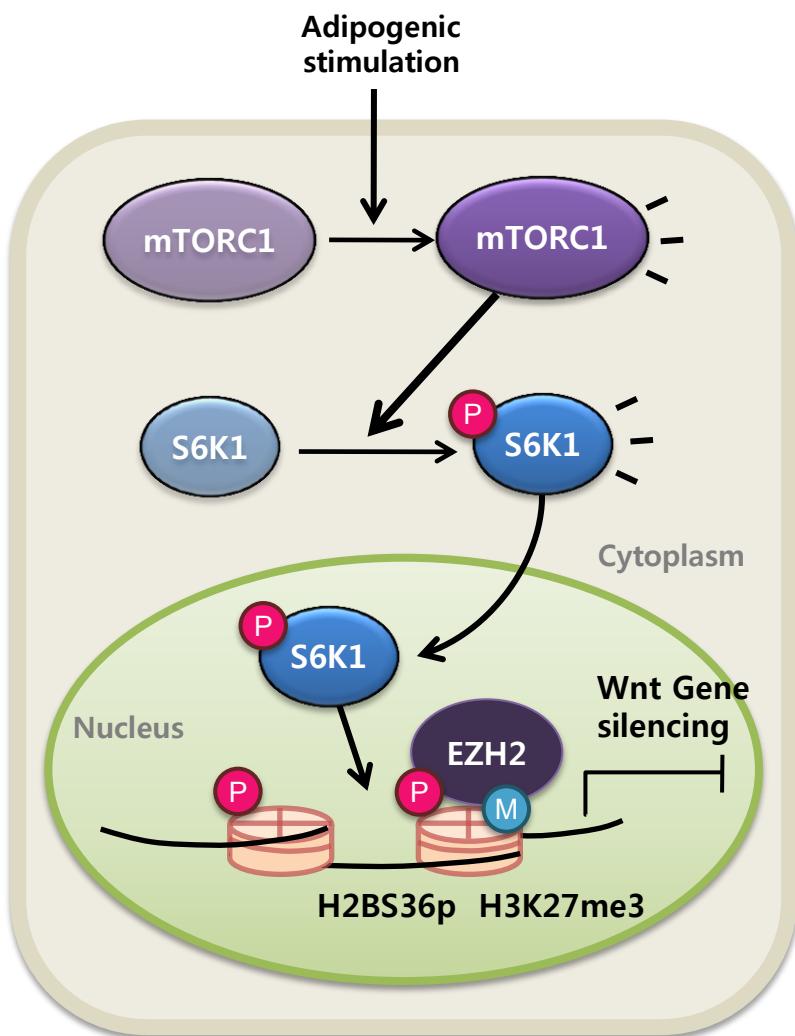
#	Height	Weight	BMI	Gfoup
1	161	75	28.93	Overweight
2	155	64.9	27.01	Overweight
3	153	56	23.92	Overweight
4	154	53	22.35	Normal
5	165	66	20.20	Normal
6	165	54.7	20.09	Normal

## Part IV

### 13. P-S6K & histone modifications in human WAT



# Summary & Conclusion



- Promotion of adipogenesis by S6K1 is mediated through the regulation of a complex epigenetic network, triggered by S6K1 nuclear translocation and H3K27 trimethylation by EZH2.
- S6K1 binds to and phosphorylates H2B at serine 36, promoting H3K27me3 by EZH2.
- H3K27me3 acts at the Wnt6, Wnt10a, and Wnt10b gene promoters to block their expression.
- In turn, inhibition of Wnt gene expression leads to upregulation of PPAR $\gamma$  and C/EBP $\alpha$ , inducing increased adipogenesis.
- Consistent with these findings white adipose tissue from S6K1-deficient mice has no detectable H2BS36p or H3K27me3, while exhibiting enhanced Wnt gene expression.
- Critically, the extent of H2BS36p and H3K27me3 from adipose tissue of mice maintained on a high fat diet or from obese humans, correlates with their body mass index.
- These findings place S6K1-dependent H2BS36p as a key regulator of H3K27me3 during the commitment of mesenchymal stem cells to the adipogenic lineage.

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**Thank You For Listening :-)**