2014 International Conference on Diabetes & Metabolism

Epigenetic regulation of adipogenesis by MLL3/MLL4 complex

Young-Wook Cho (조 영 욱)

Korea Basic Science Institute (한국기초과학지원연구원)

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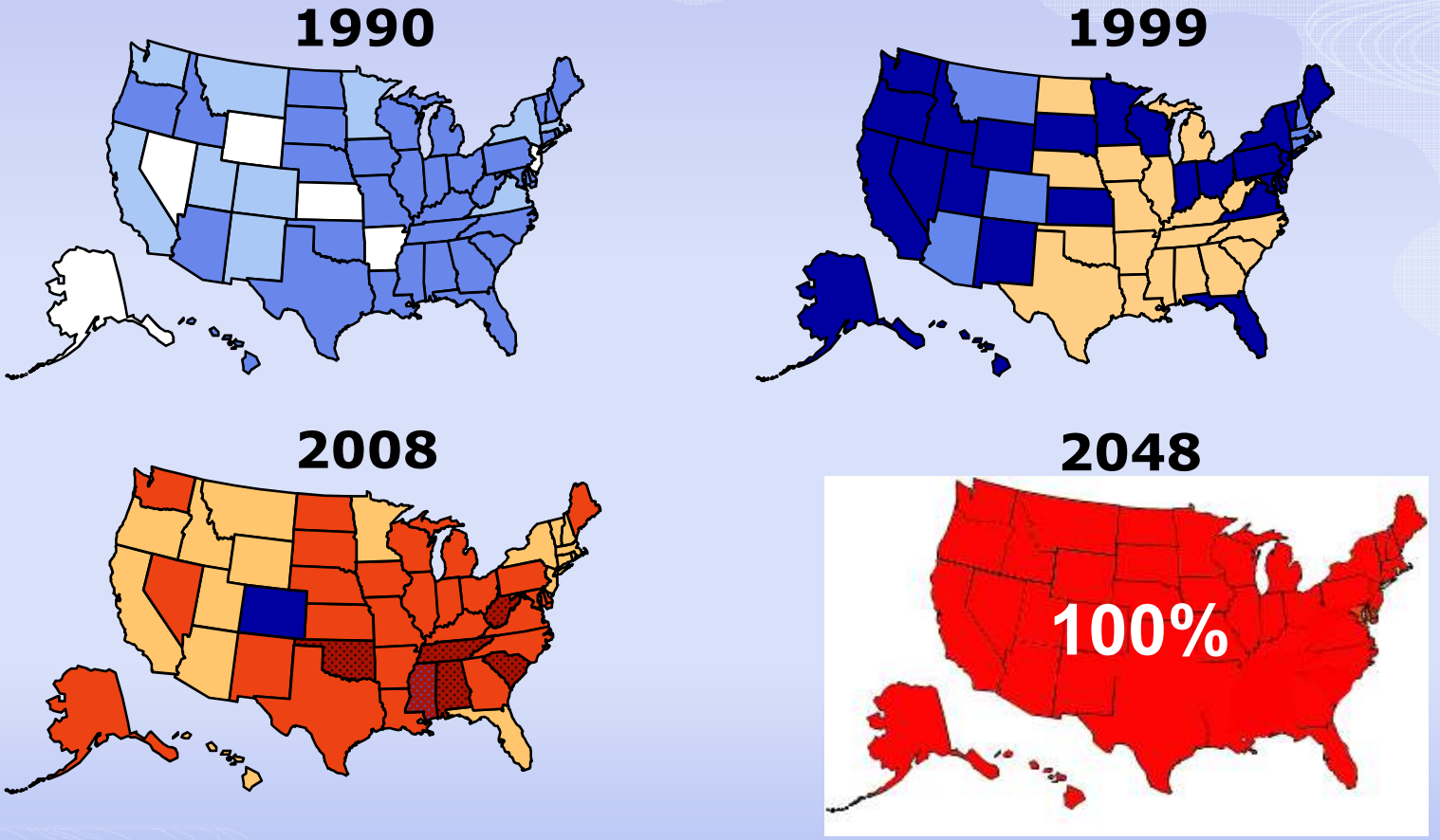
Part I

Introduction

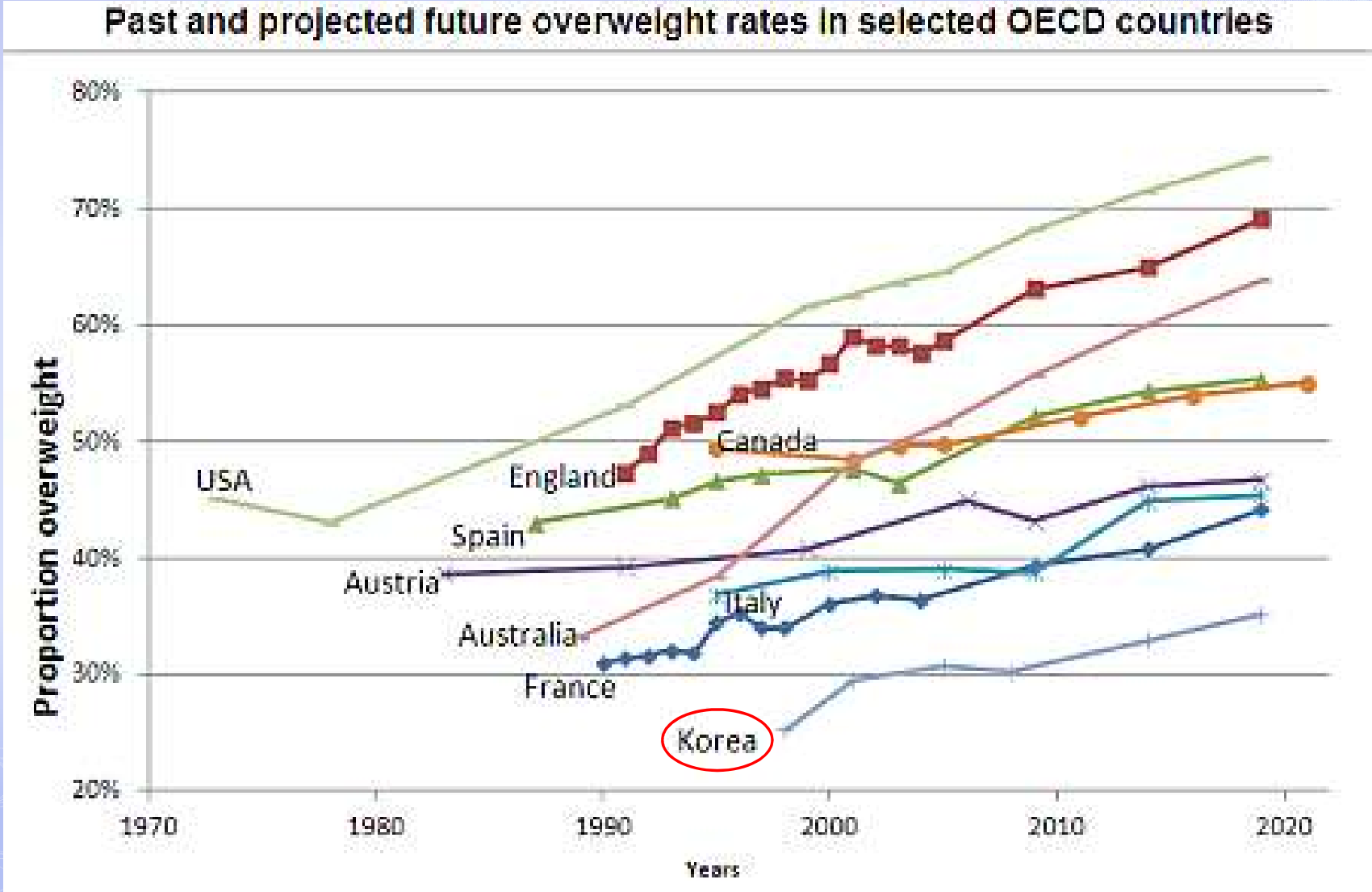
Obesity Trends among U.S. Adults

(*BMI ≥30; weight(kg)/height(m)^2)



CDC

Global Obesity Epidemic

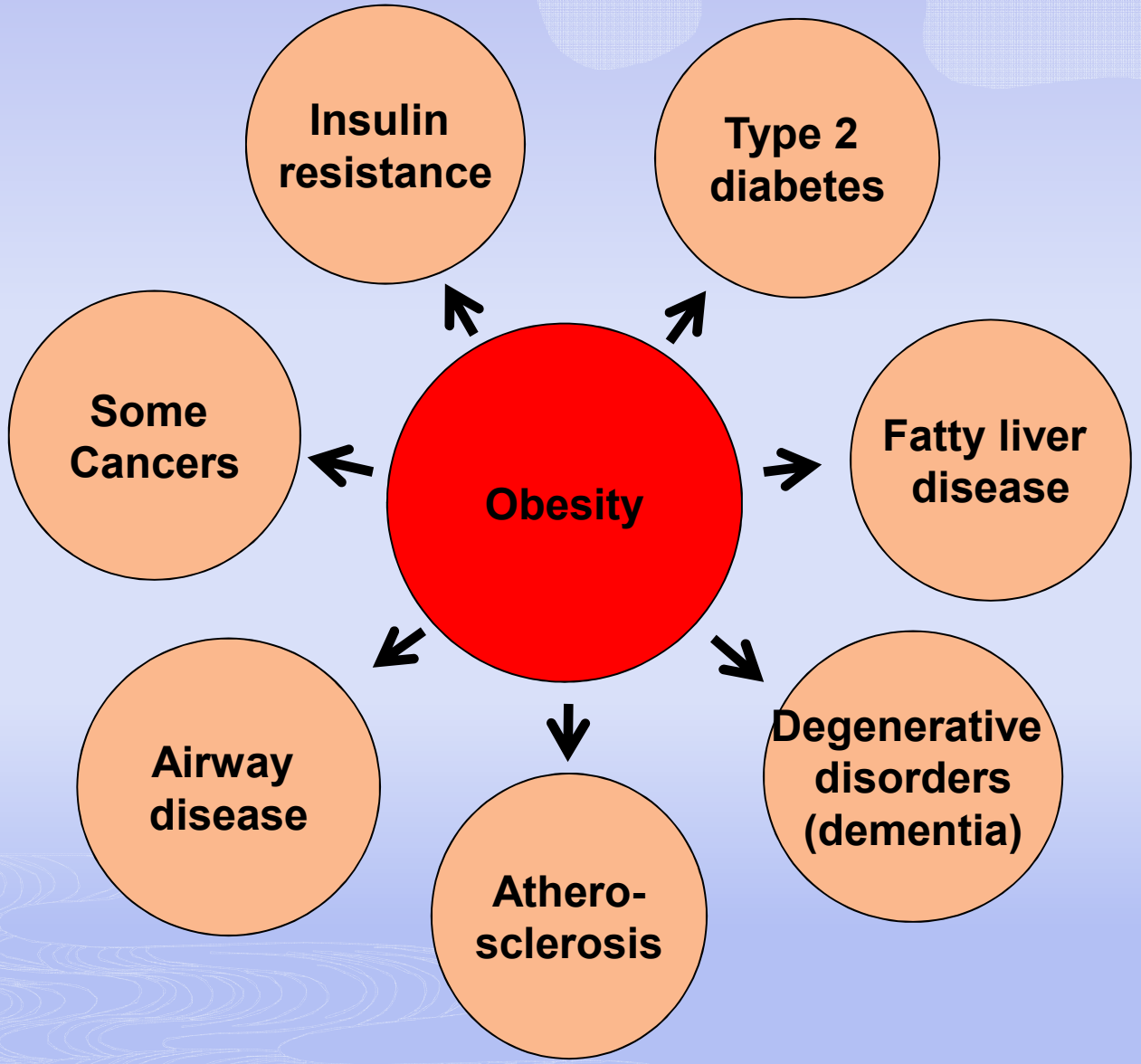


2009 OECD

What Cause Obesity?

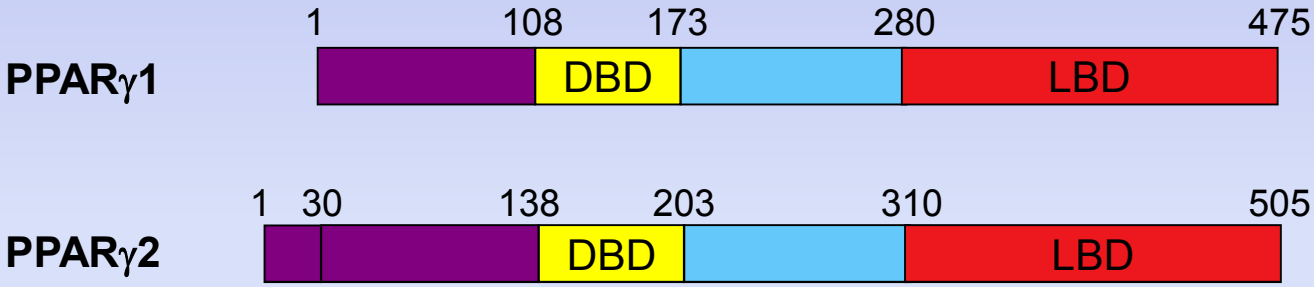
- Overeating
- Physical inactivity
- Slow metabolism
- Genetics - leptin deficiency
- Lack of Sleep
- Medications
- Psychological factors
- Diseases – Cushing's disease
- Gut microbes

Obesity-Related Health Problems



Peroxisome Proliferator-Activated Receptor γ (PPARγ)

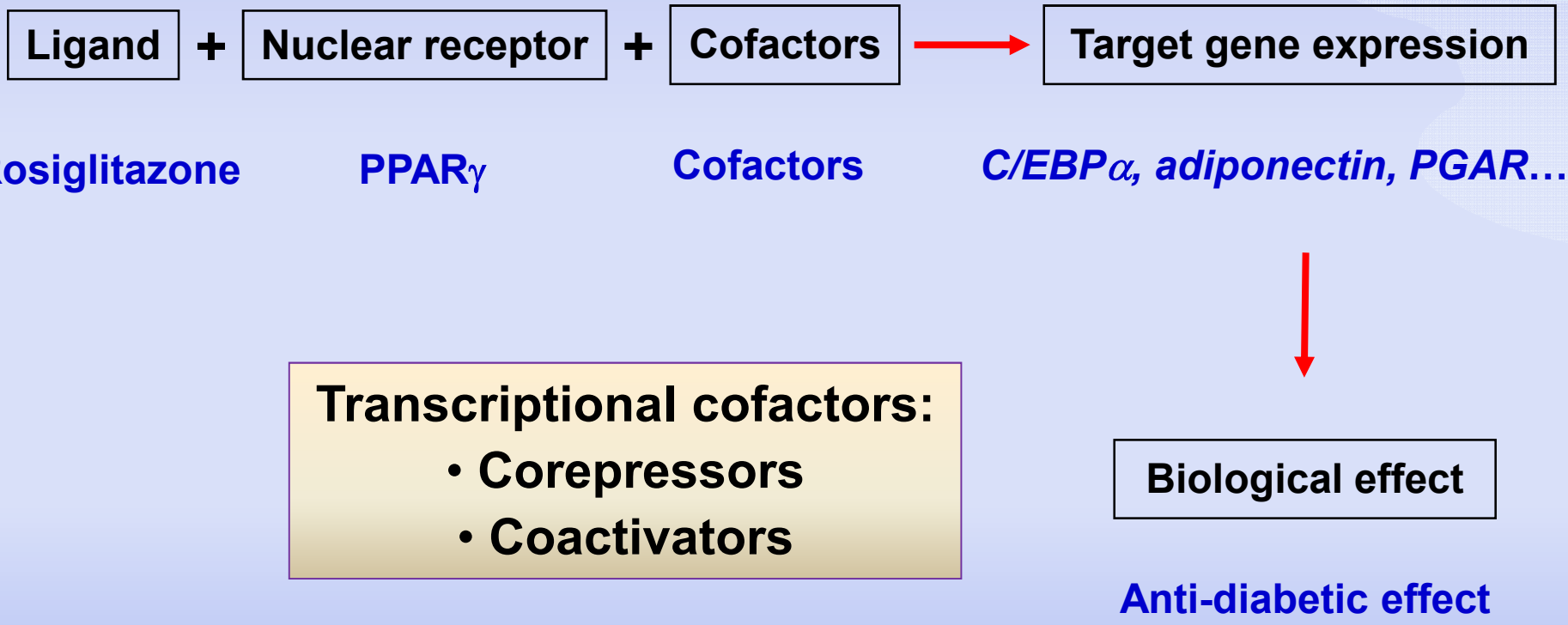
- Nuclear receptor family of ligand-binding transcription factor



- **Master regulator of adipogenesis**
  - Both γ1 & γ2 are highly expressed in adipocytes; both are important for adipogenesis
  - Non-adipogenic mouse embryonic fibroblasts $\xrightarrow{\text{PPARγ}}$ adipocytes

- **Drug target for type II diabetes**
  - Synthetic PPARγ ligands, e.g. Rosiglitazone, were used for millions of type II diabetic patients

Molecular Mechanisms of Nuclear Receptor Biology



Transcriptional Cofactors

On naked DNA:

- **Mediator complex: binds RNA polymerase II and nuclear receptors**

On chromatin (epigenetics):

- **ATP-dependent chromatin remodeling complexes**
- **Histone modifying complexes: acetylation, methylation…**

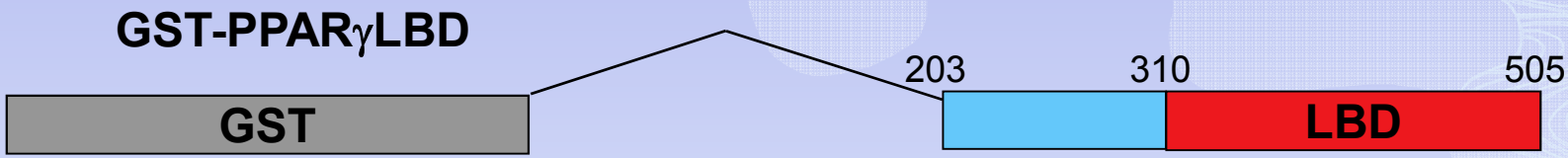
Methylation on histone H3 lysine 4 (H3K4): gene activation

Methylation on histone H3 lysine 27 (H3K27): gene repression

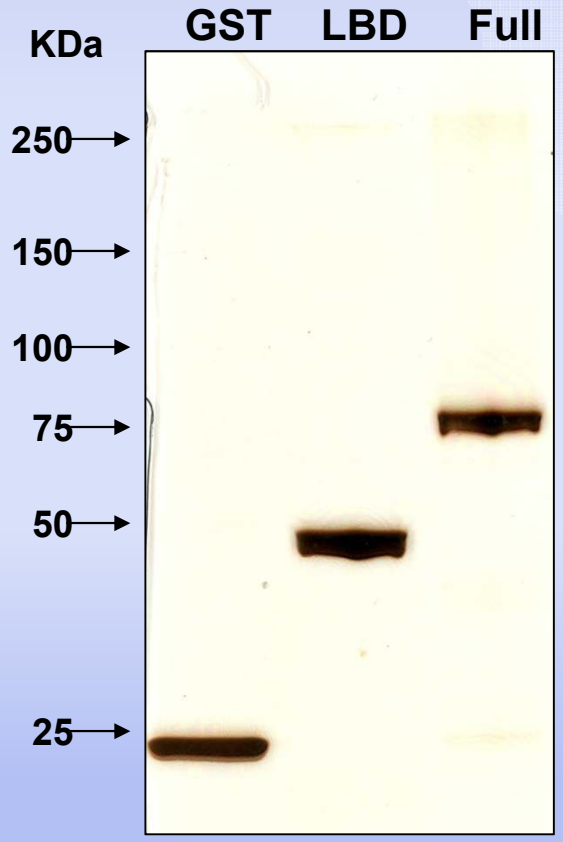
Part II

MLL3/MLL4 complex has both histone H3K4 methyltransferase activity and histone H3K27 demethylase activity

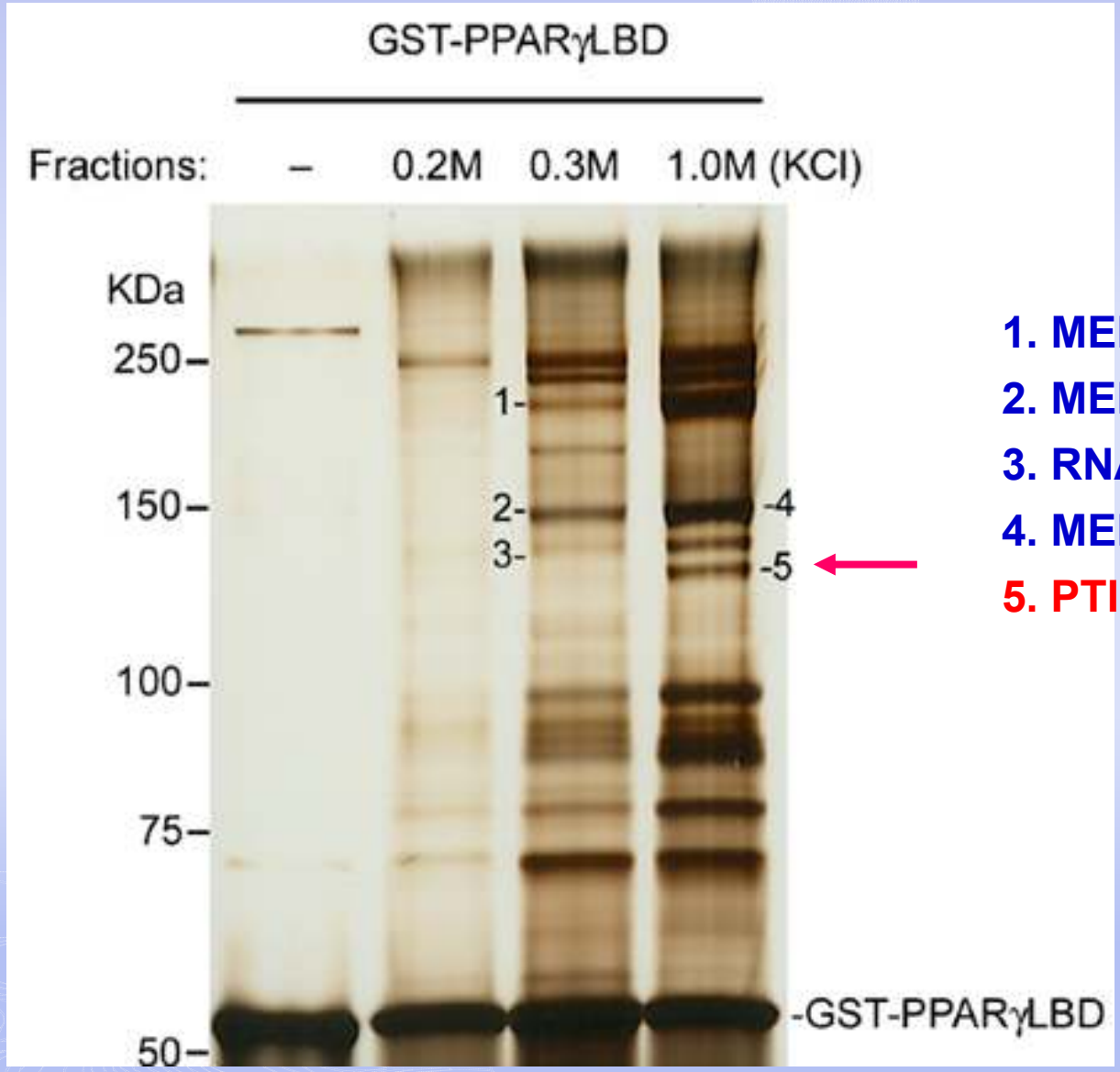
GST-PPARγLBD fusion protein



- Nuclear Extract from HeLa
- Bio-Rex 70 column
- Fractions of 0.2M, 0.3M and 1.0M KCl
- Dialyzing against 0.1M KCl
- Incubated with GST-PPARγ-LBD
- Elute PPARγ binding proteins
- Run SDS-PAGE
- Identified by Mass Spectrometry



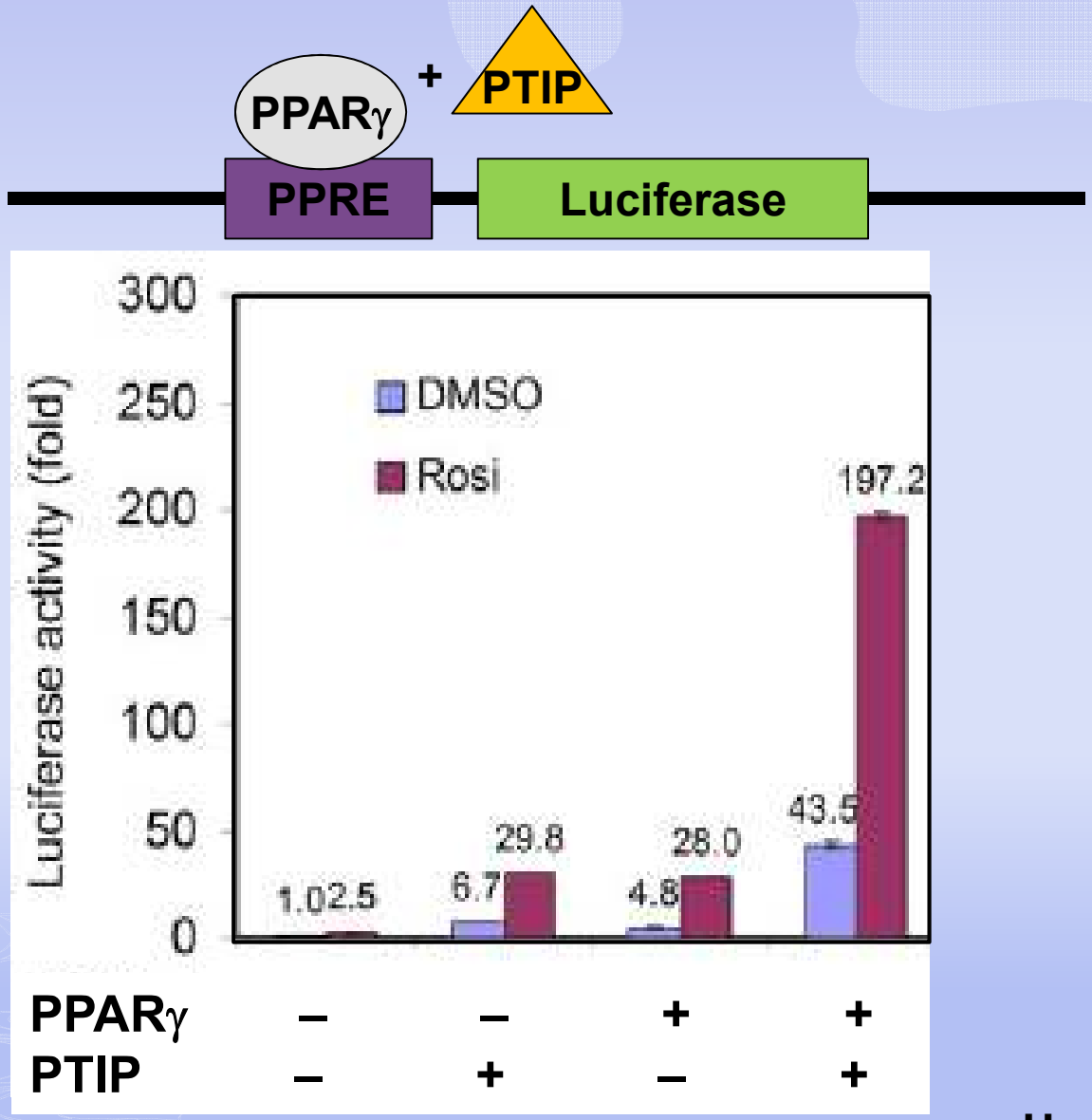
PPARγ Binding Proteins



1. MED1 / TRAP220
2. MED23 / Sur2
3. RNA Pol II subunit 2
4. MED14 / TRAP170
5. PTIP (Pax transactivation domain-interacting protein)

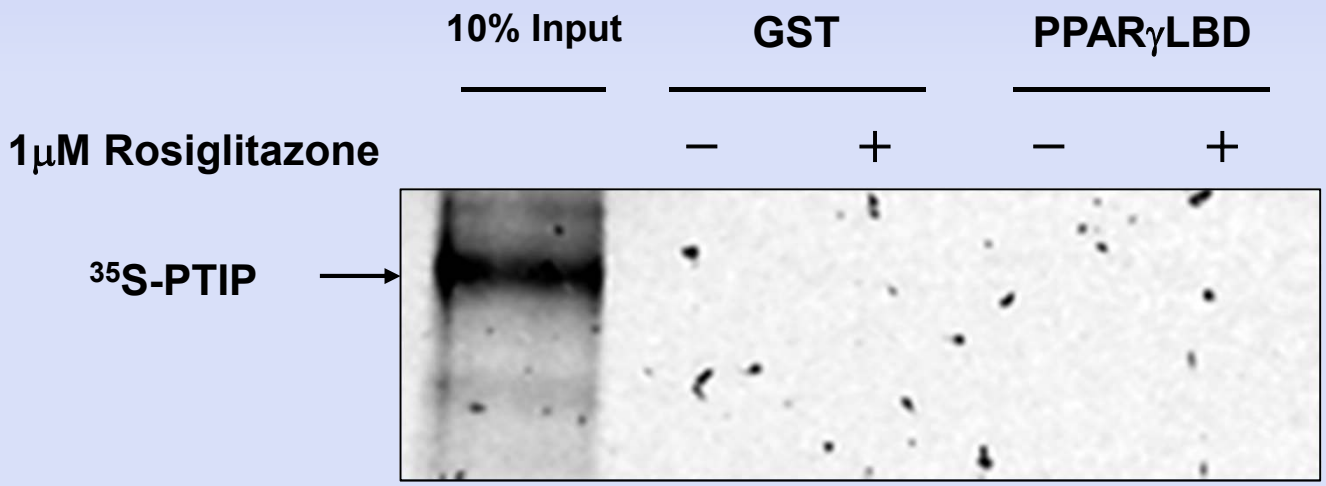
Unpublished data

PTIP is a Novel Transcriptional Coactivator of PPARγ



Unpublished data

Lack of Interaction between PTIP & PPARγ *in vitro*

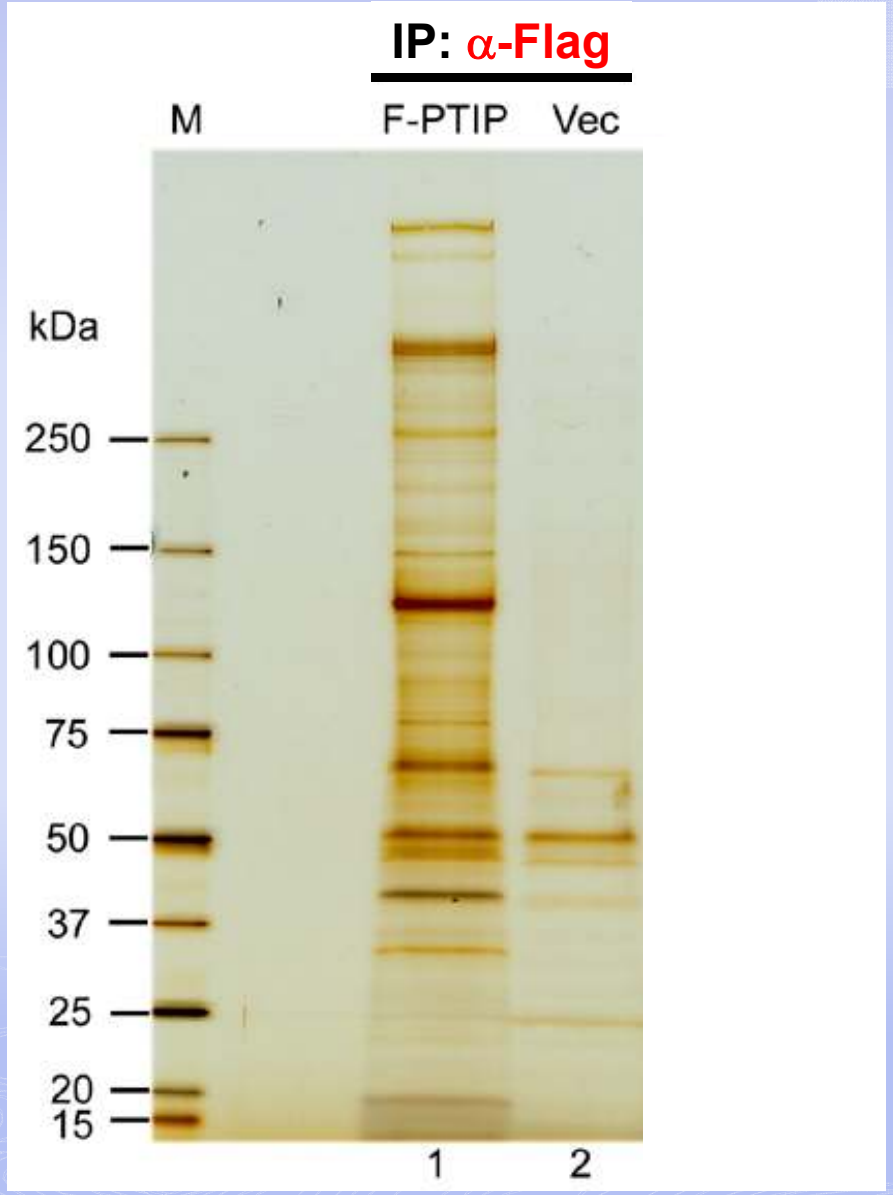


Unpublished data

Pax Transactivation domain-Interacting Protein (PTIP)

- A ubiquitously expressed nuclear protein
- Carries 6 **BRCT** domains that are predominantly found in proteins involved in DNA damage response
- In response to ionizing radiation, ectopic PTIP binds 53BP1 and translocates to DNA damage-induced foci (*Science 2003)*
- **Normal cellular function of PTIP was unclear**
- PTIP is important for immunoglobulin class switch recombination(*Science 2010*)

Immunoprecipitation of Flag-PTIP



- **Human Set1-like HMT complexes:**
  MLL4, MLL3, NCOA6, ASH2L, RBBP5, WDR5, hDPY-30

- **F-PTIP, PA1, UTX**

- **DNA damage response & repair:**
  53BP1, MRE11, RAD50, BLM

Purification of MLL3/MLL4 Complex

HeLaS/Flag-PTIP nuclear extracts

↓

1st IP: α-Flag antibody

↓

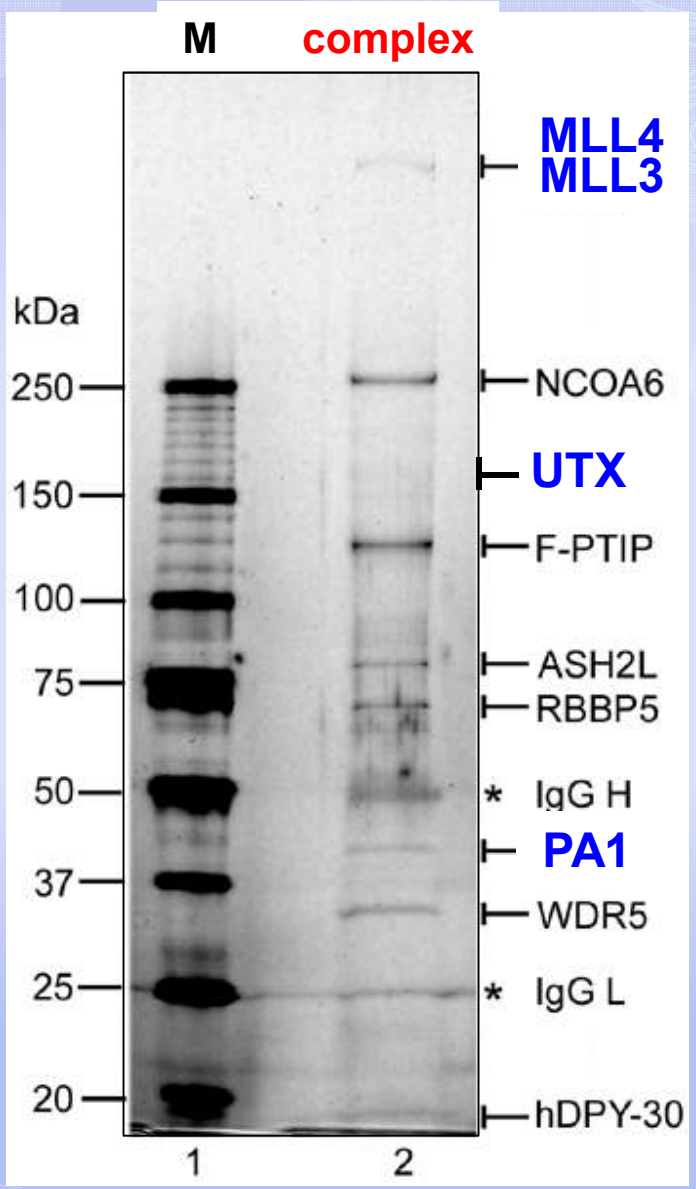
PTIP-associated proteins

↓

2nd IP: α-RbBP5

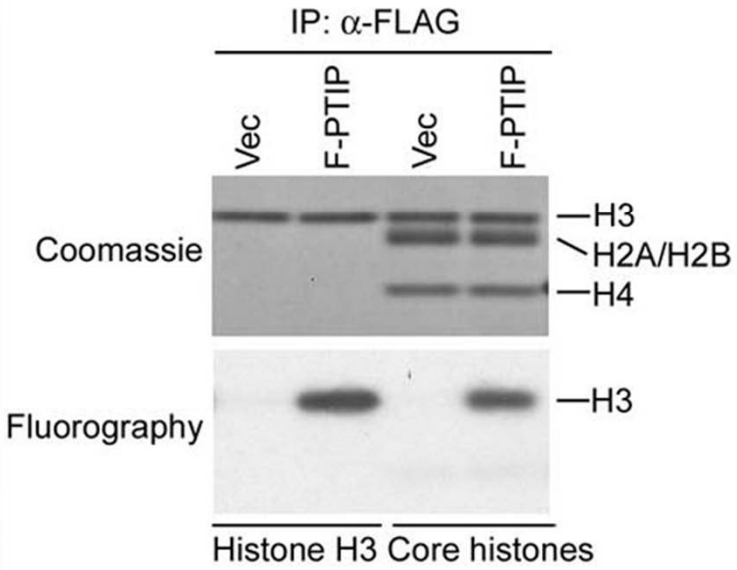
↓

MLL3/MLL4 complex

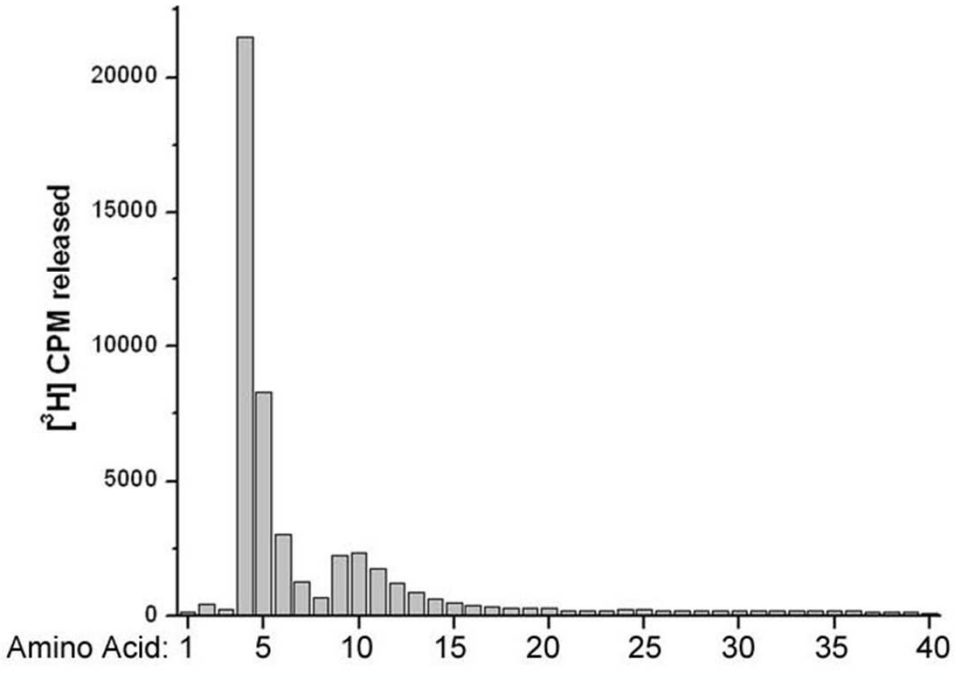


MLL3/MLL4 Complex Methylates Histone H3 on K4

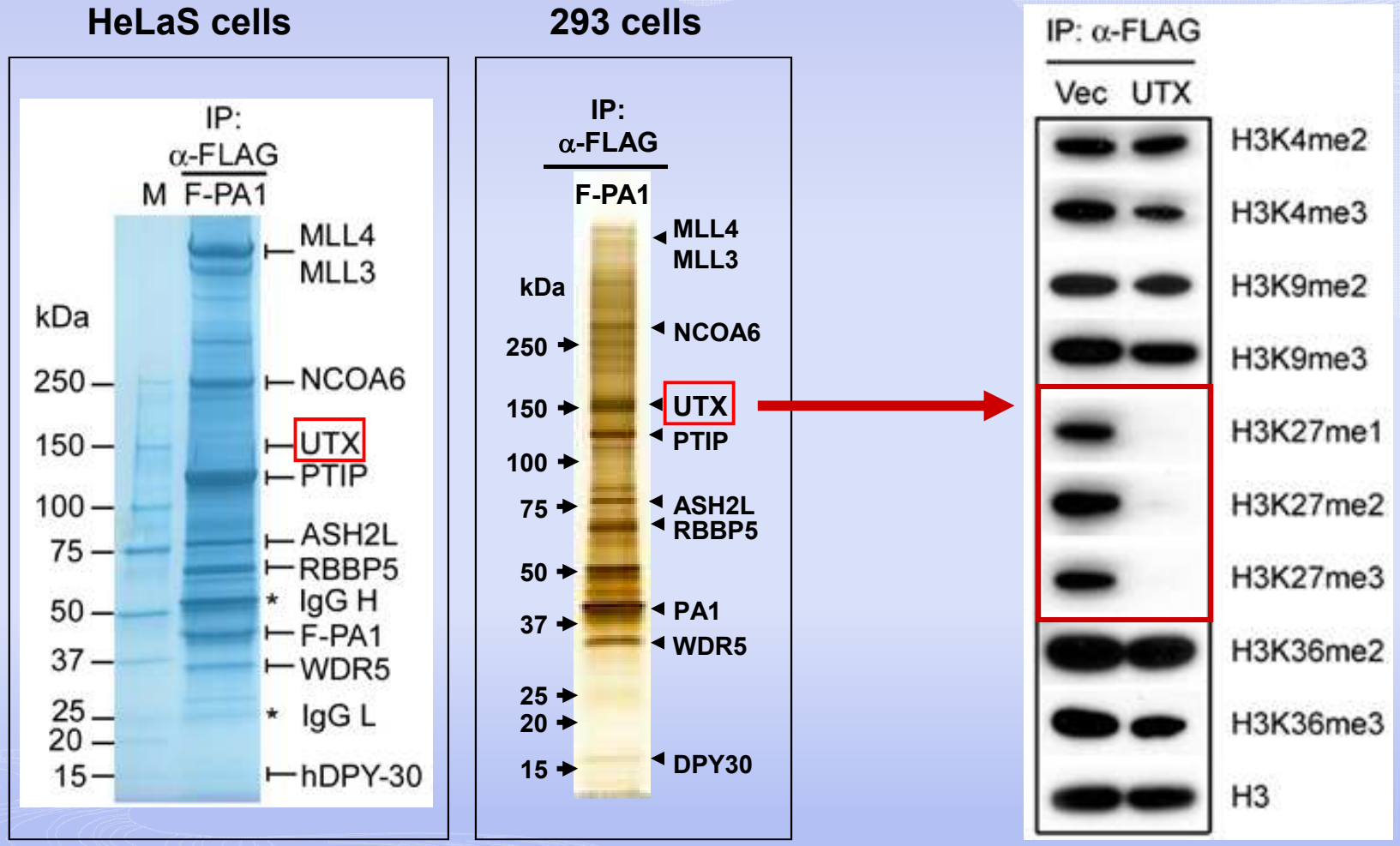
A. HMT assay:



B. Edman degradation of methylated Histone H3:

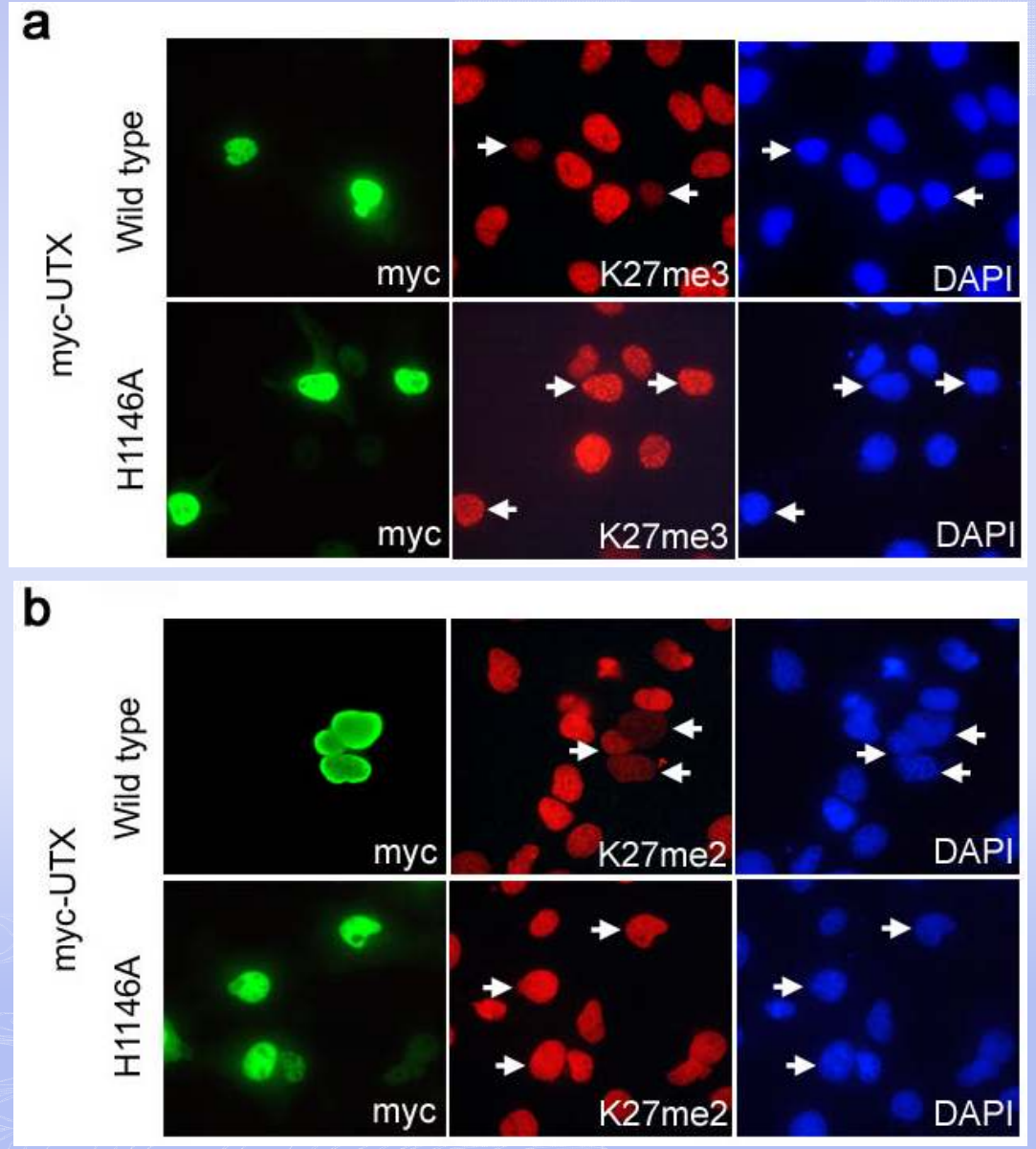


UTX is a Histone H3K27 Demethylase



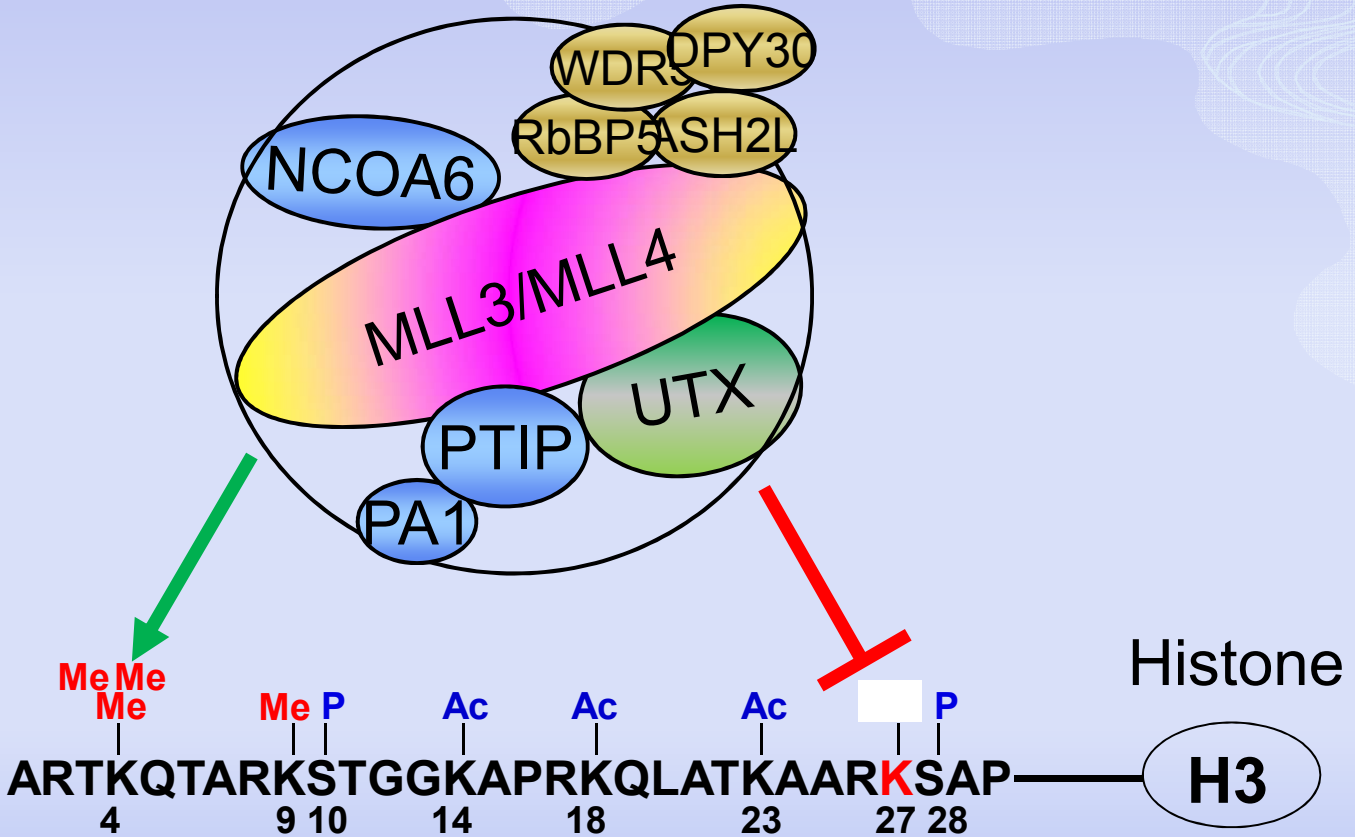
PNAS (2007)

UTX expression results in reduced H3K27 in Cos7



PNAS

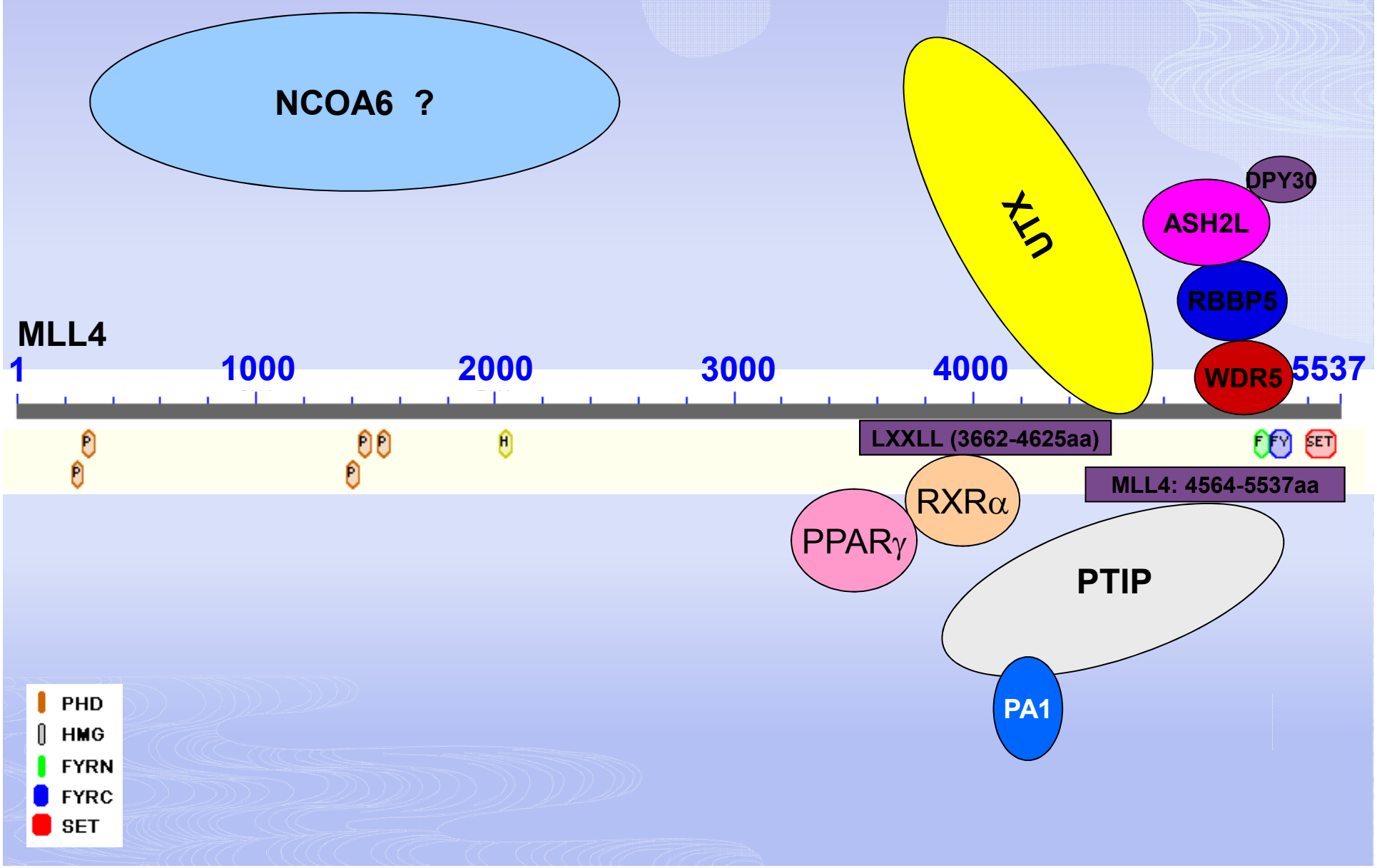
MLL3/MLL4 Complex Mediated Histone Modification



Methylation on histone H3 lysine 4 (H3K4): gene activation

Methylation on histone H3 lysine 27 (H3K27): gene repression

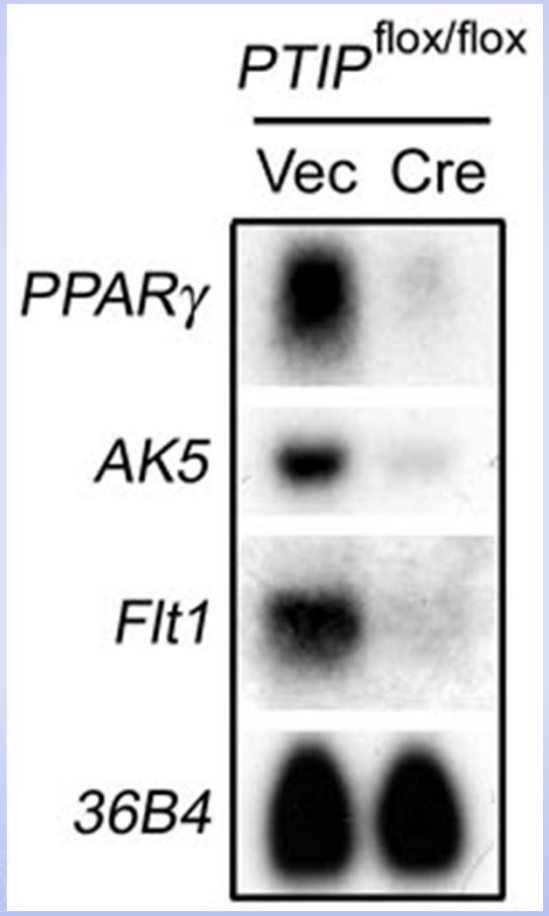
Interaction among MLL3/MLL4 Complex Subunits

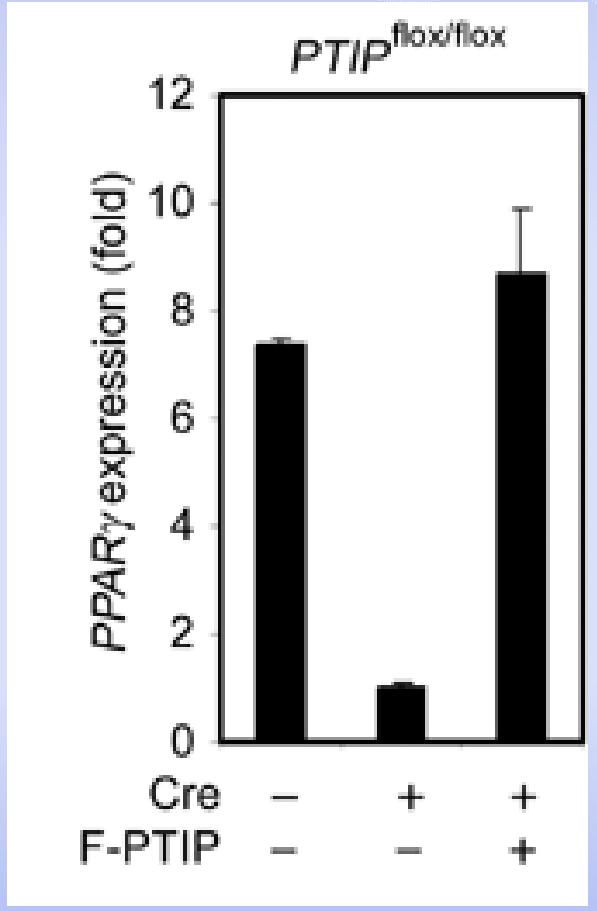


Part III

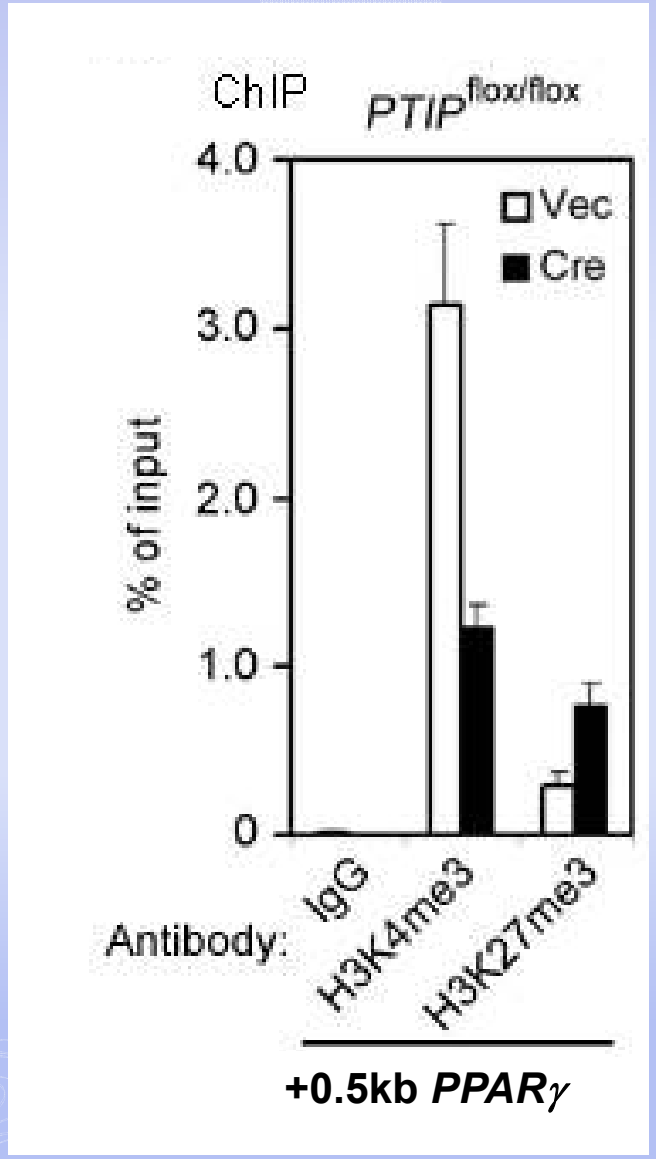
MLL3/MLL4 complex is required for adipogenesis

MLL3/MLL4 Complex Regulates PPARγ Expression

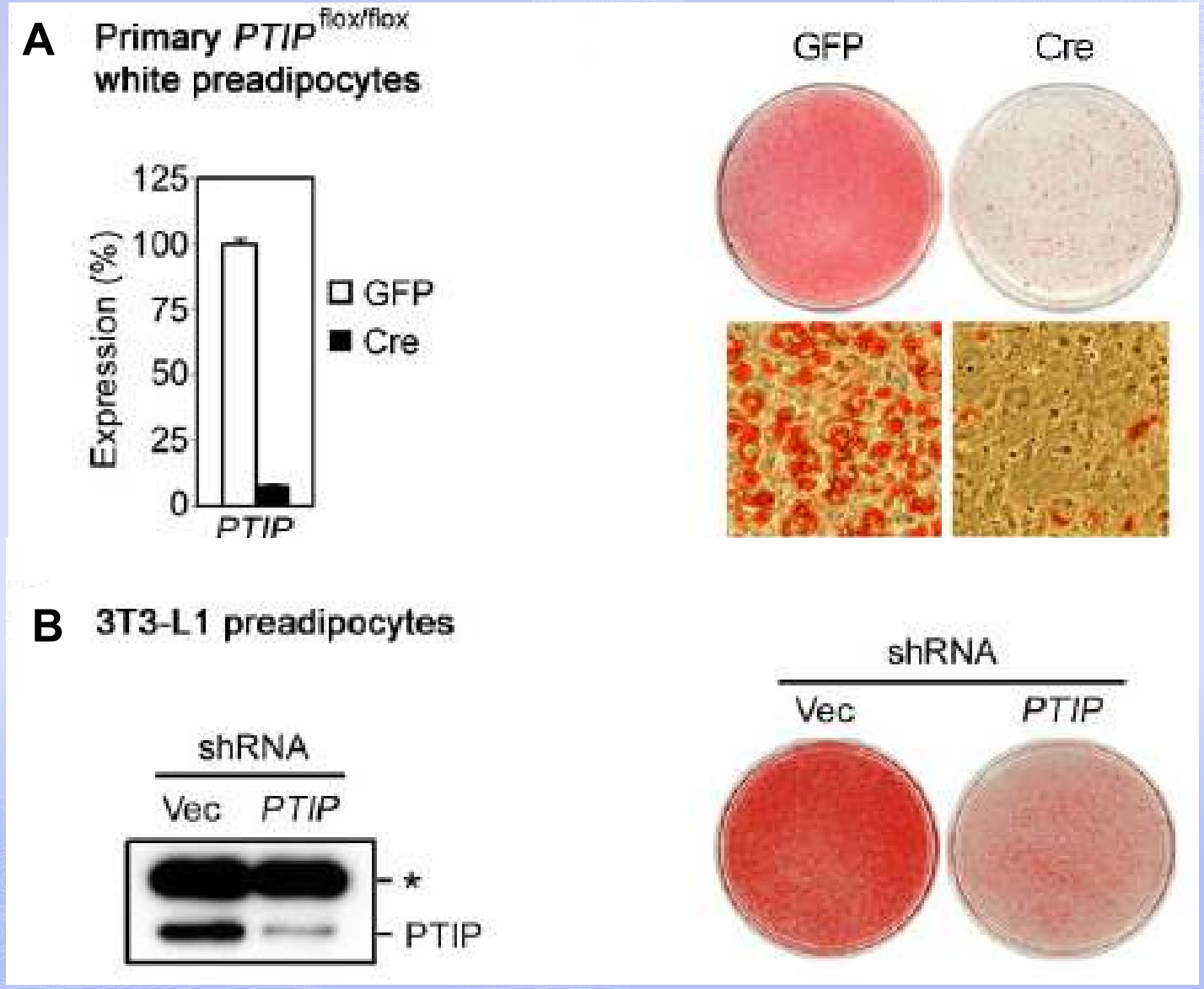




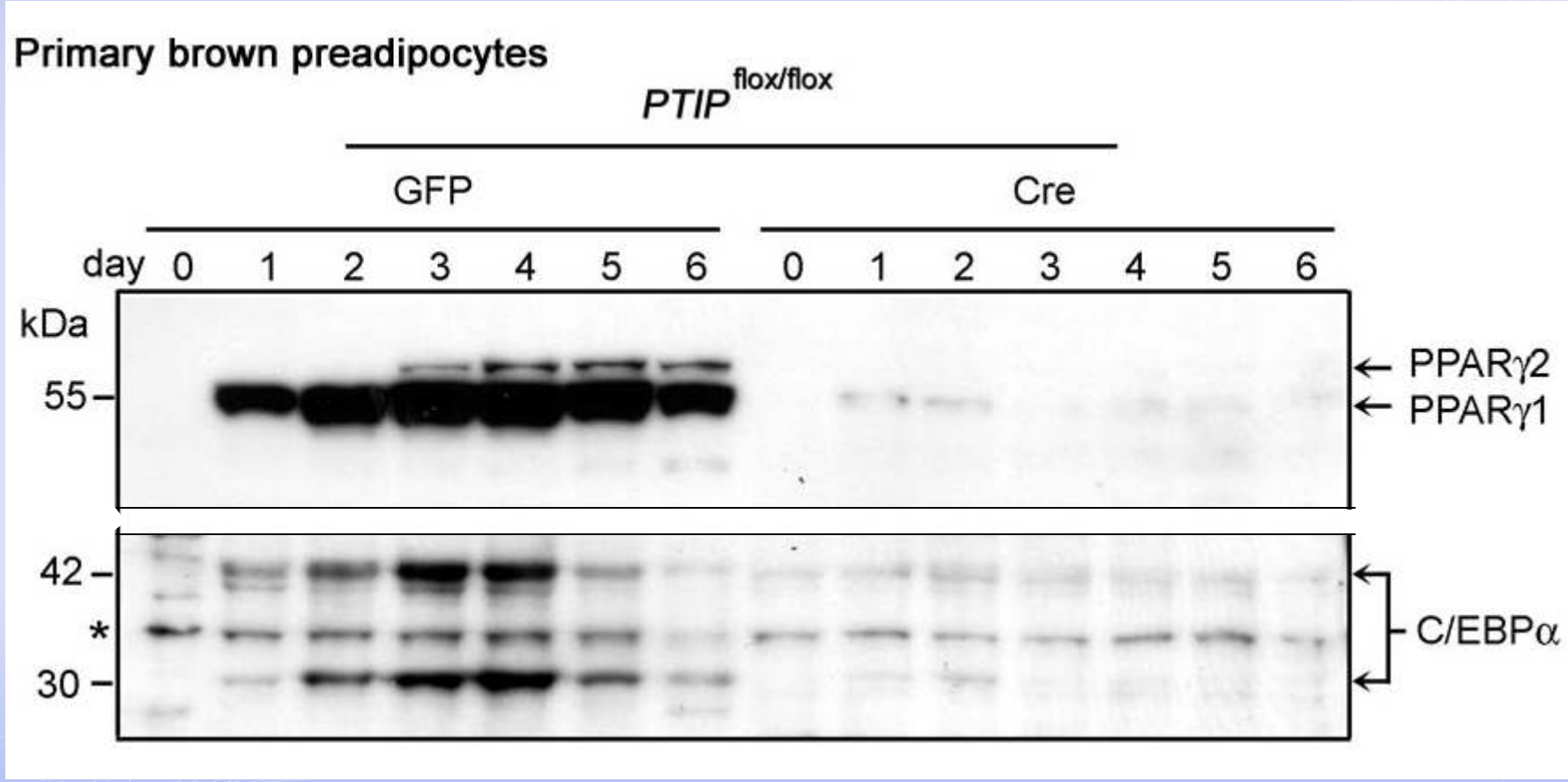
Histone Methylations on PPARγ



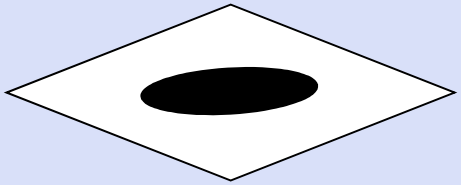
PTIP Complex is Required for Adipogenesis



PPARγ and CEBPα Expression



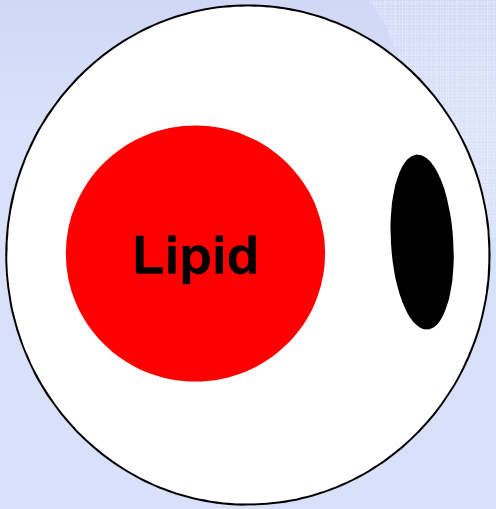
In vitro Adipogenesis



Mouse embryonic fibroblast

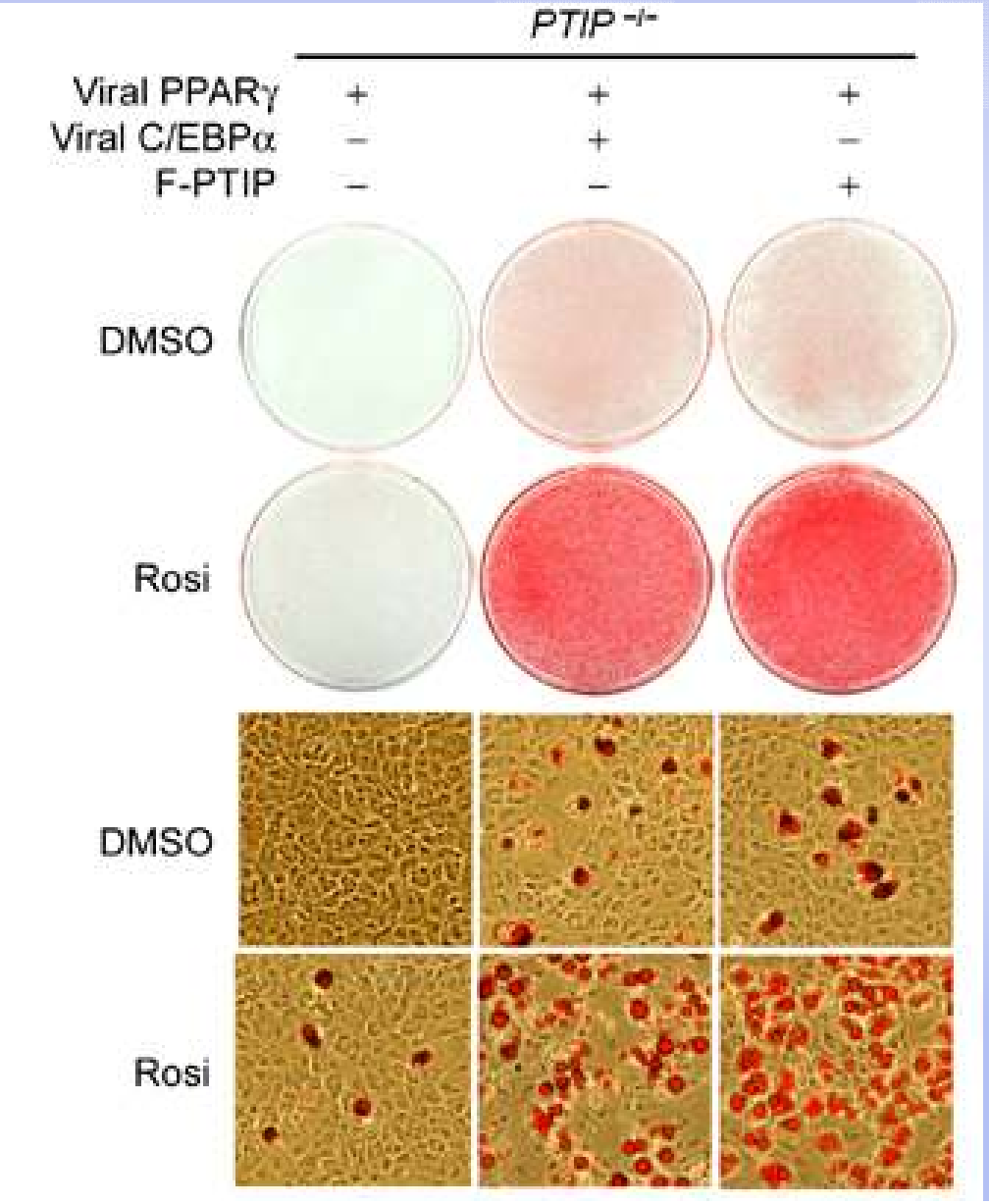
Ectopic Expression
PPARγ or C/EBPα

Differentiation stimuli:
- Dexamethasone
- cAMP
- Insulin
- Growth factors

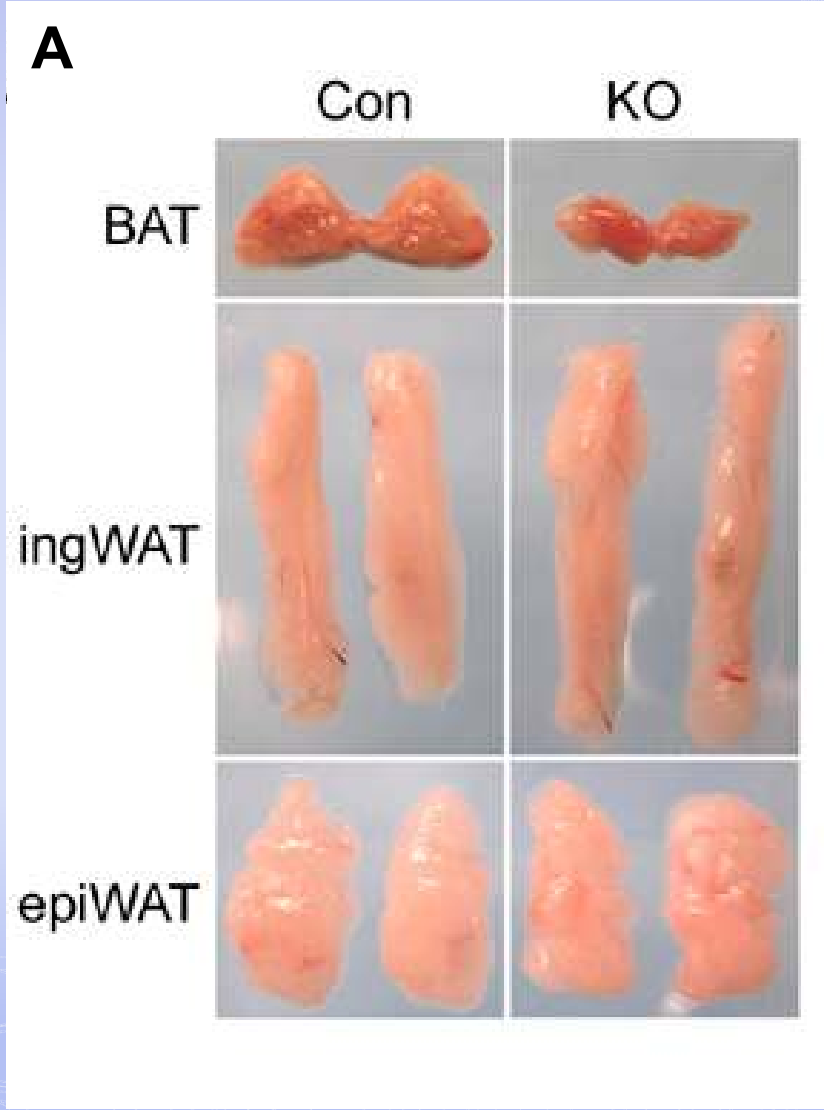


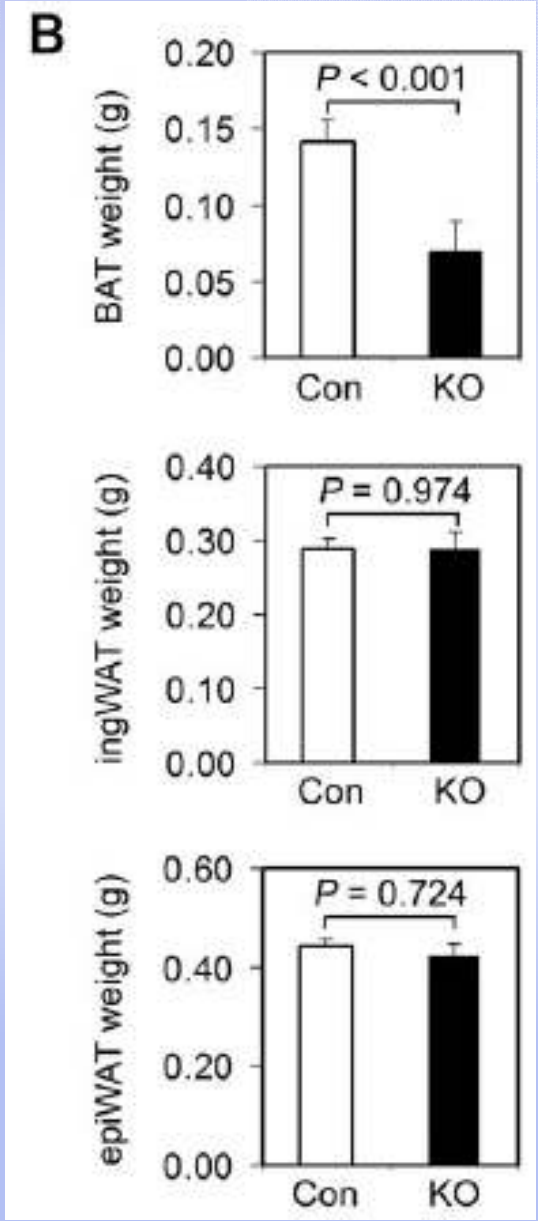
Adipocyte

Adipogenesis in PTIP KO Cells



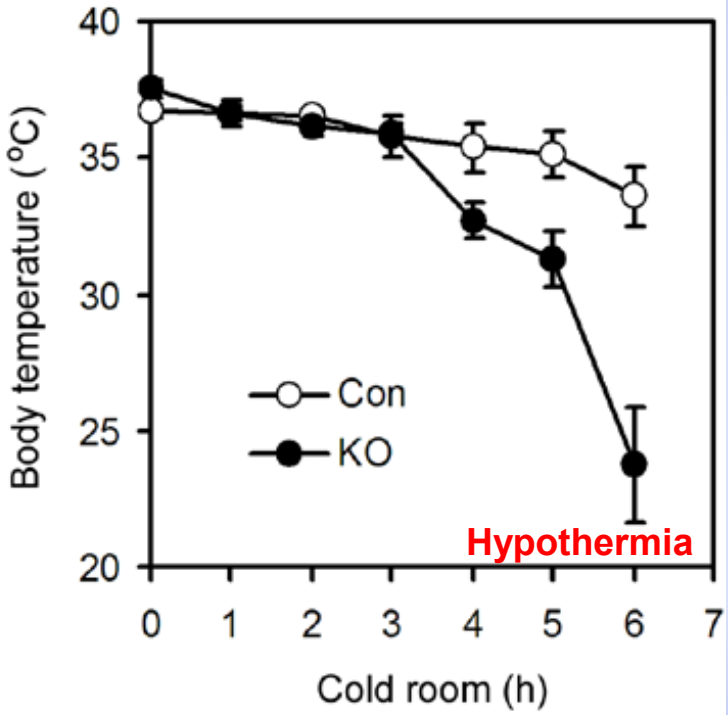
Adipose Tissue-Specific PTIP KO mice



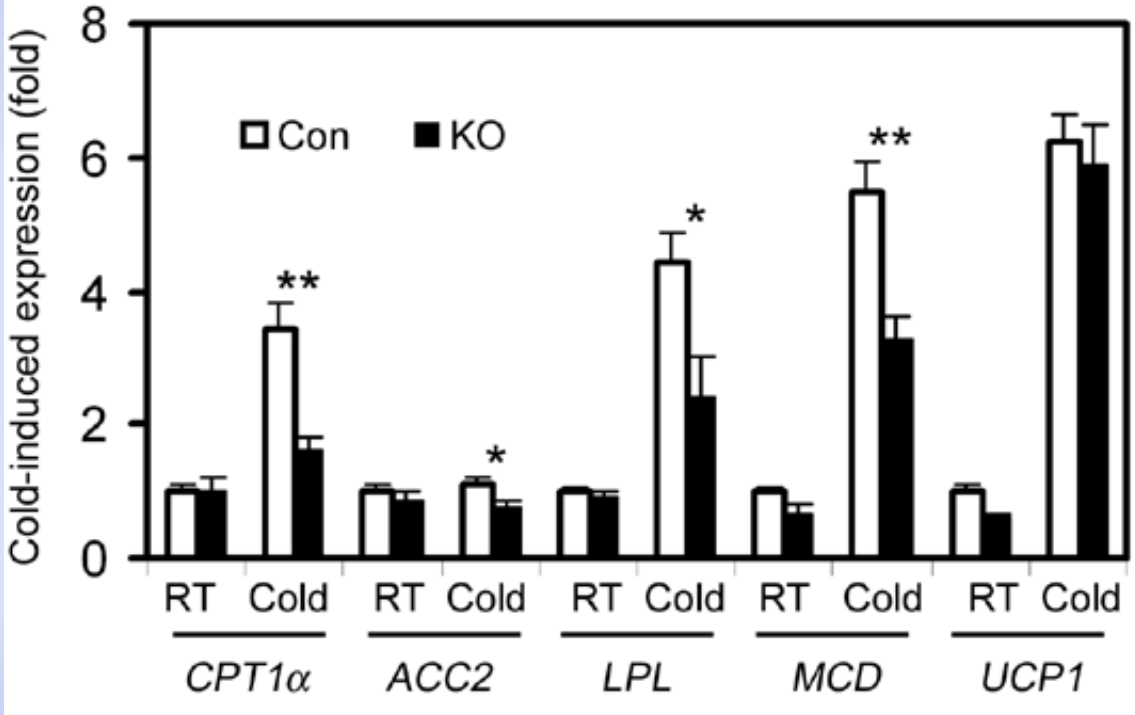


Cold Intolerance

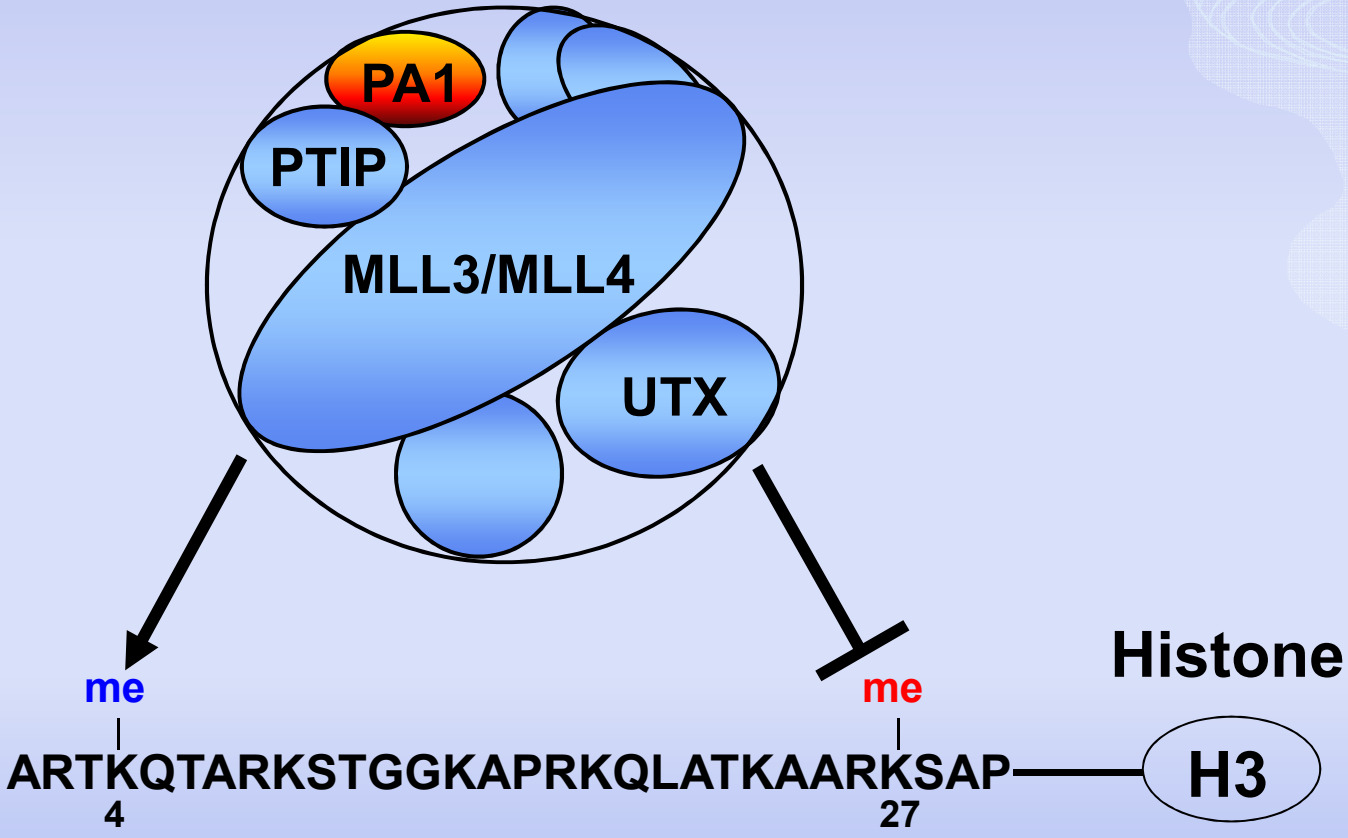
Cold tolerance



qRT-PCR



PA1 in MLL3/MLL4 Complex



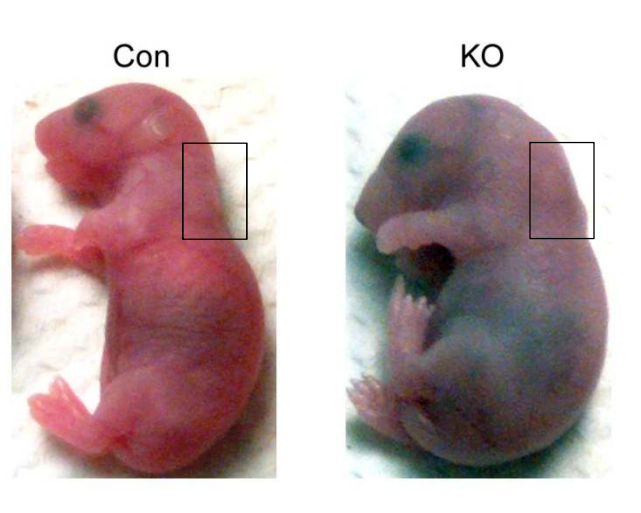
Methylation on H3K4: gene activation

Methylation on H3K27: gene repression

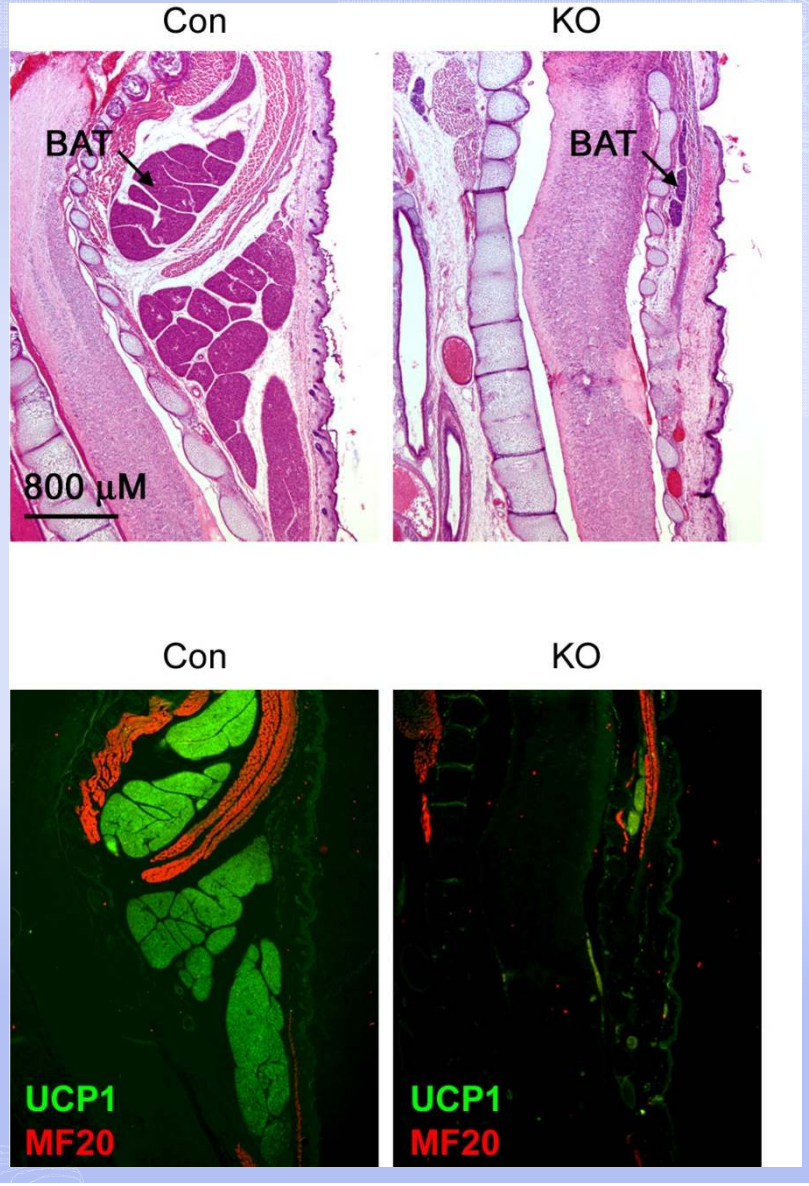
Brown Fat-Specific PA1 Knockout Mice

Mating: $PA1^{lox/lox}$ x $PA1^{lox/+}$ Myf5-Cre

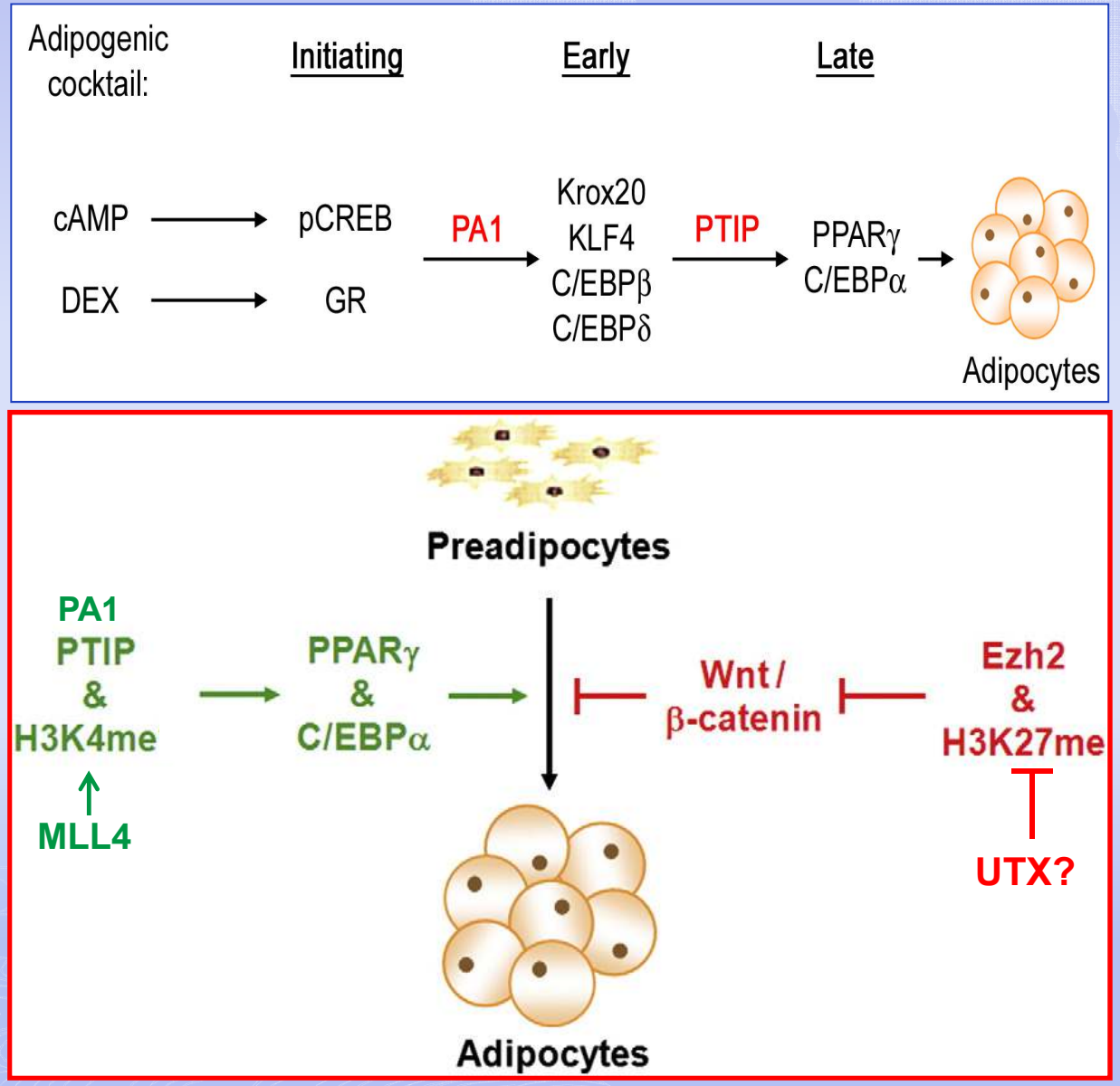
E18.5



Genotype (E18.5)	Number of embryos	Survival (%)
PA1$^{lox/+}$	15	100
PA1$^{lox/+}$; *Myf5-Cre*	15	100
PA1$^{lox/lox}$	12	100
PA1$^{lox/lox}$; *Myf5-Cre*	11	0

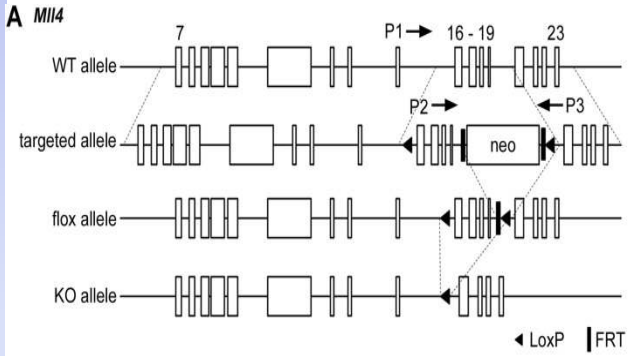


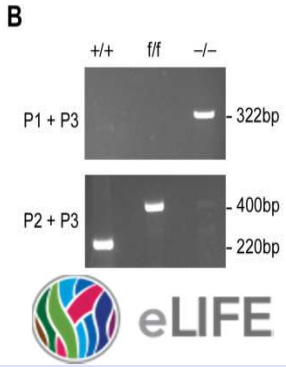
Regulation of Adipogenesis by MLL3/MLL4 Complex



Gene deletion prevents high-fat diet induced fatty liver

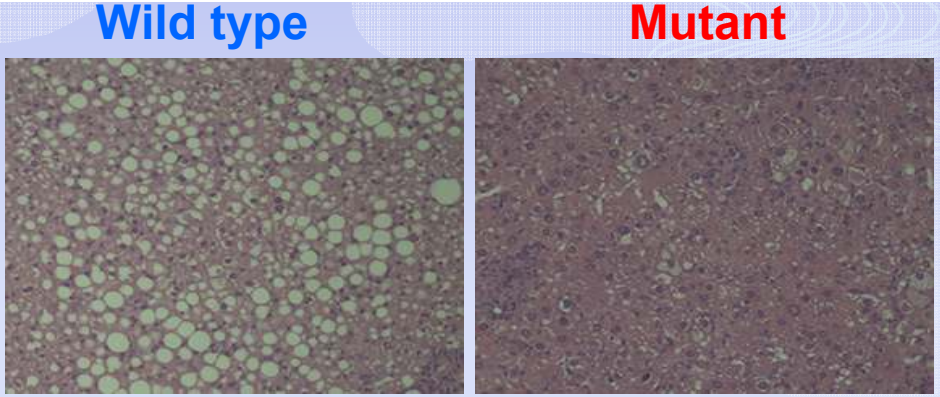
1) Conditional knockout mouse



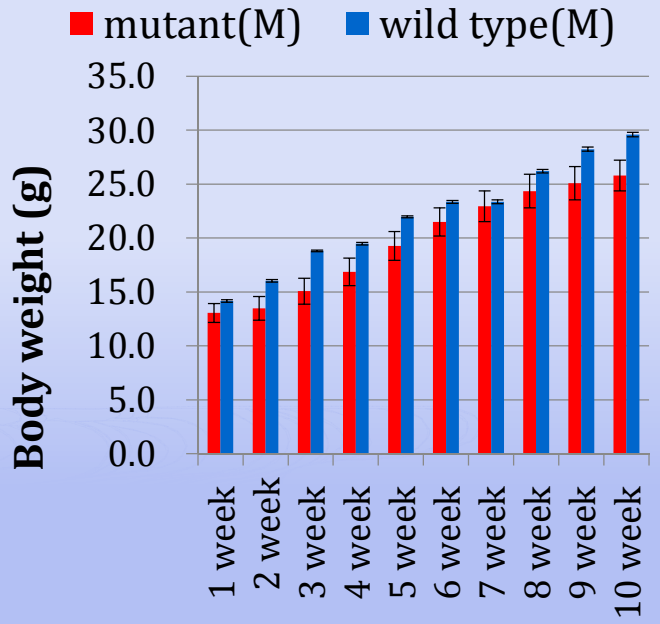


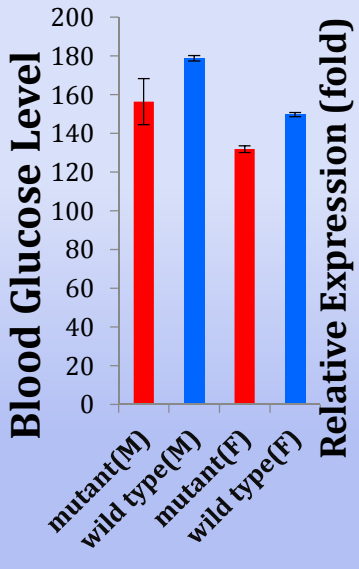
eLife 2013;2:e01503

2) High-fat diet induced fatty liver

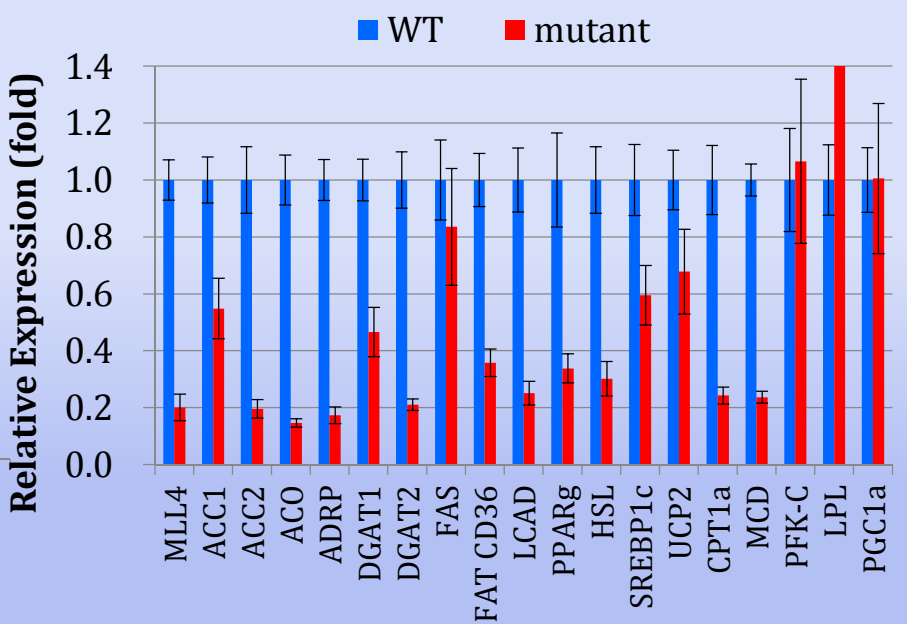


3) Body weight and Blood glucose

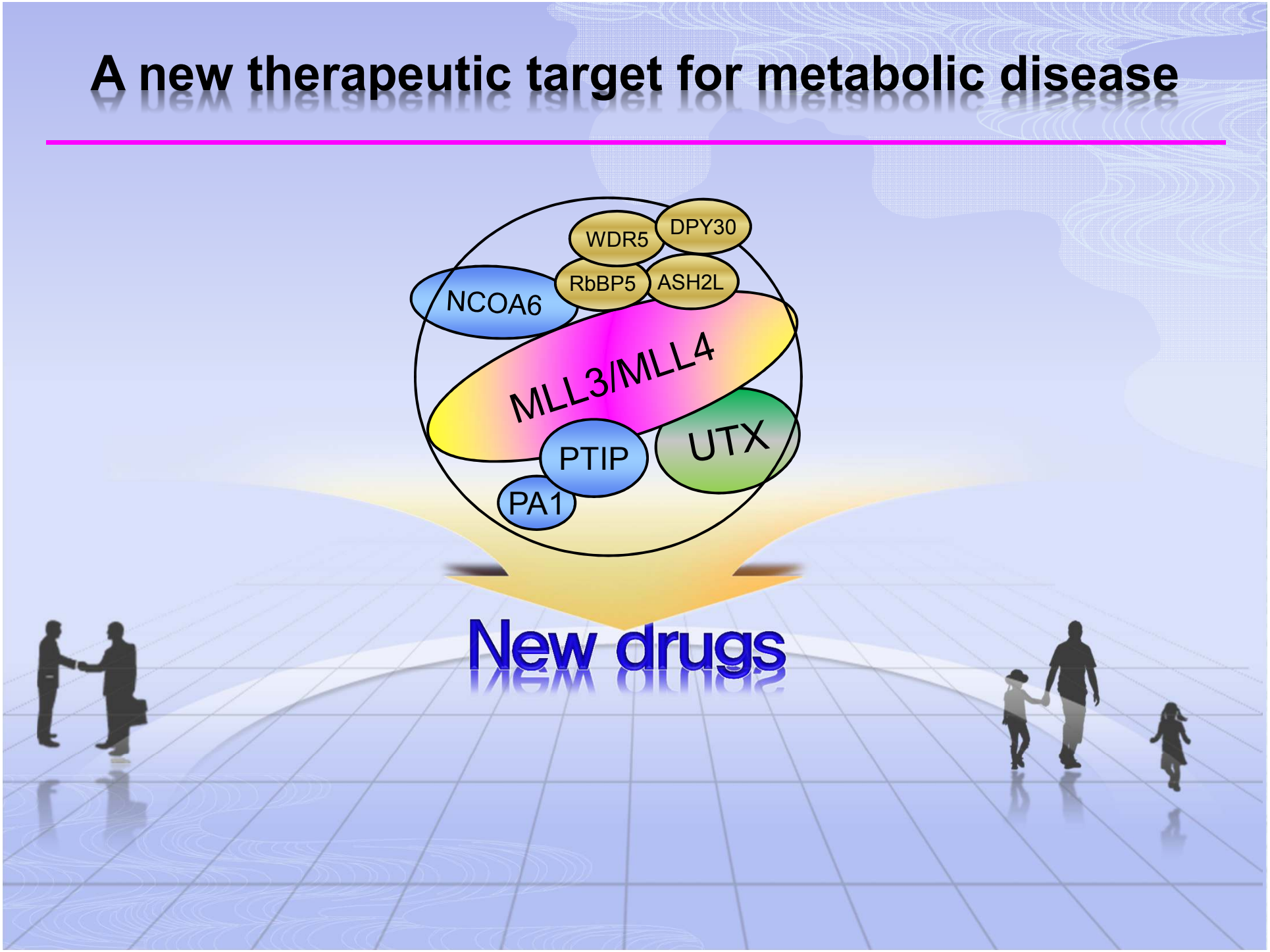




4) mRNA expression



A new therapeutic target for metabolic disease



Acknowledgement

Korea Basic Science Institute
(Chuncheon center)
HaNa Gu

The National Institute of
Diabetes and Digestive and
Kidney Diseases (NIDDK)
Kai Ge
SunHwa Hong
Qihuang Jin
Lifeng Wang
Chaochen Wang
Ji-Eun Lee

The best equipments, scientists and technologies

Thank you

KBSI 한국기초과학지원연구원
KOREA BASIC SCIENCE INSTITUTE

SET1 & SET1-Like Complexes

Yeast Set1 Complex	hSet1 Complex	MLL Complex	MLL2 Complex	MLL3/MLL4 Complex	
Set1	hSet1	MLL	MLL2	MLL3 & MLL4	HMT with SET domain
Bre2	ASH2L	ASH2L	ASH2L	ASH2L	Common subunits
Swd1	RBBP5	RBBP5	RBBP5	RBBP5	
Swd3	WDR5	WDR5	WDR5	WDR5	
Sdc1	hDPY30	hDPY30	hDPY30	hDPY30	
Swd2	hSwd2	-	-	-	Distinct subunits
Spp1	CXXC1	-		-	
	HCF1	Menin	Menin	NCOA6	
		HCF1		PTIP & PA1	
				UTX	
+++	+++	+++	+++	+++	H3K4 HMT activity

Histone Lysine Methylations

Methyltransferases

ASH1			SET2		
SET1A	SUV39H1		NSD1		
SET1B	SUV39H2				
MLL1	G9a				
MLL2	EuHMTase				
MLL3	SETDB1				
MLL4	CLL8				
MLL5	RIZ1	EZH2	SMYD2	DOT1L	

ARTKQTARKSTGGKAPRKQLATKAARKSAPATGGVKKPH…K… — **H3**

4 9 27 36 79

LSD1	LSD1/AR	UTX	JHDM1a	?
SMCX	JHDM2a	JMJD3	JHDM1b	
SMCY	JHDM2b		JMJD2A	
RBP2	JMJD2A		JMJD2B	
PLU-1	JMJD2B		JMJD2C	
	JMJD2C			
	JMJD2D			

Demethylases

Modified from Yi Zhang. Cell (2007)