당뇨병학회 춘계학술대회: 10 May 2013, Jeju

Leptin/AMPK Signaling in Glucose-sensitive Regulation of K_{ATP} channel Trafficking in Pancreatic β-cells

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Department of Physiology

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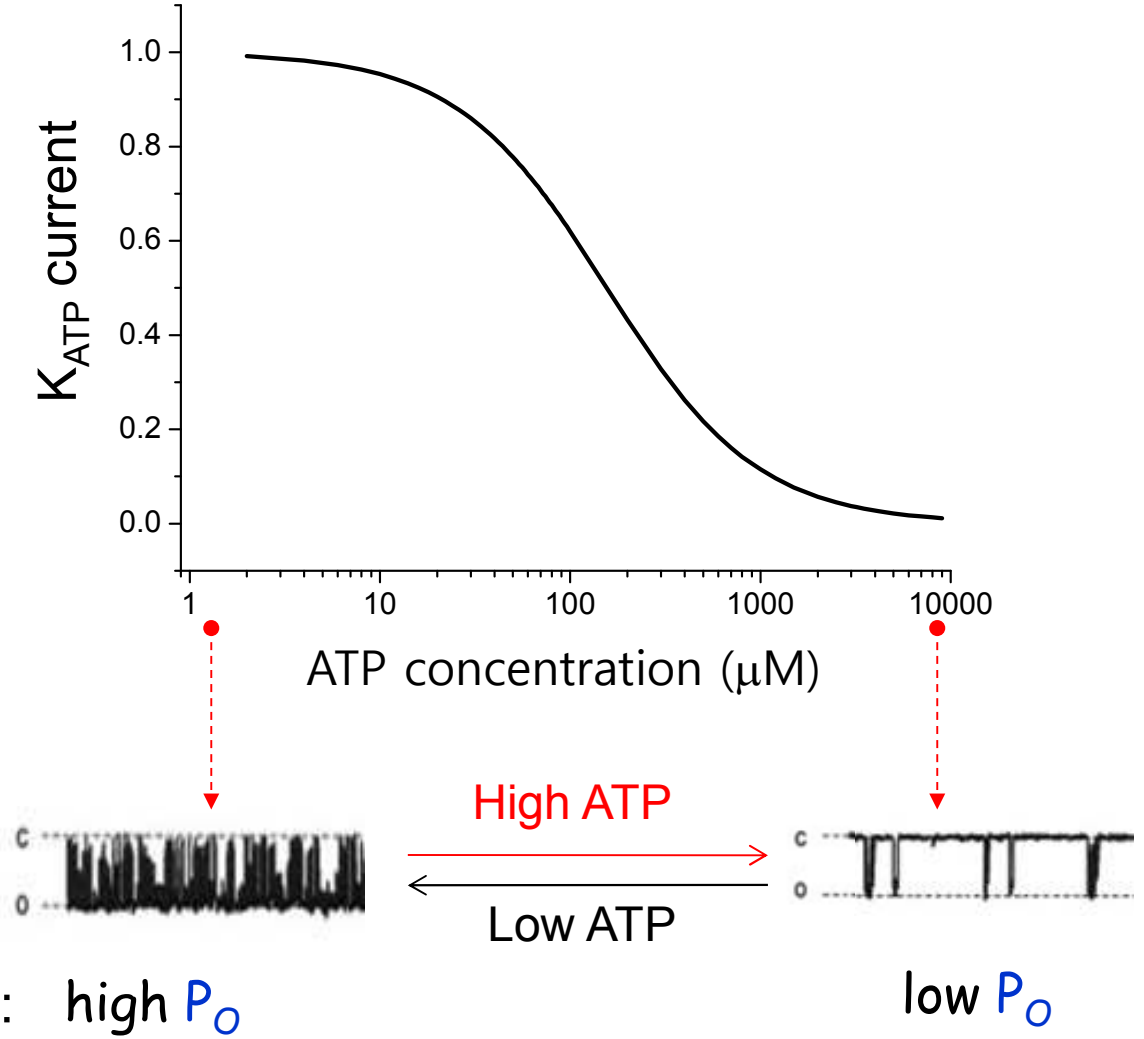
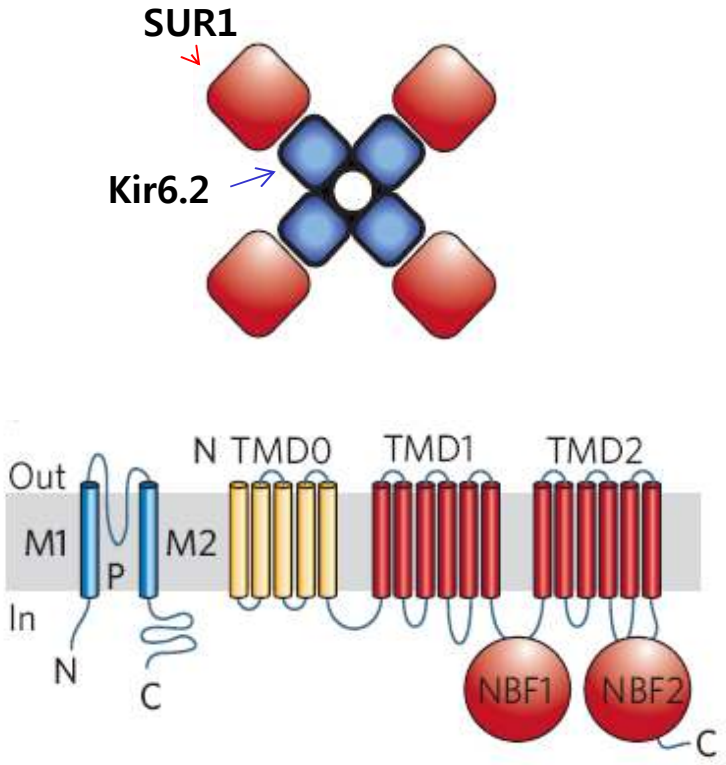
K_{ATP} channels: Molecular sensors of cellular metabolism

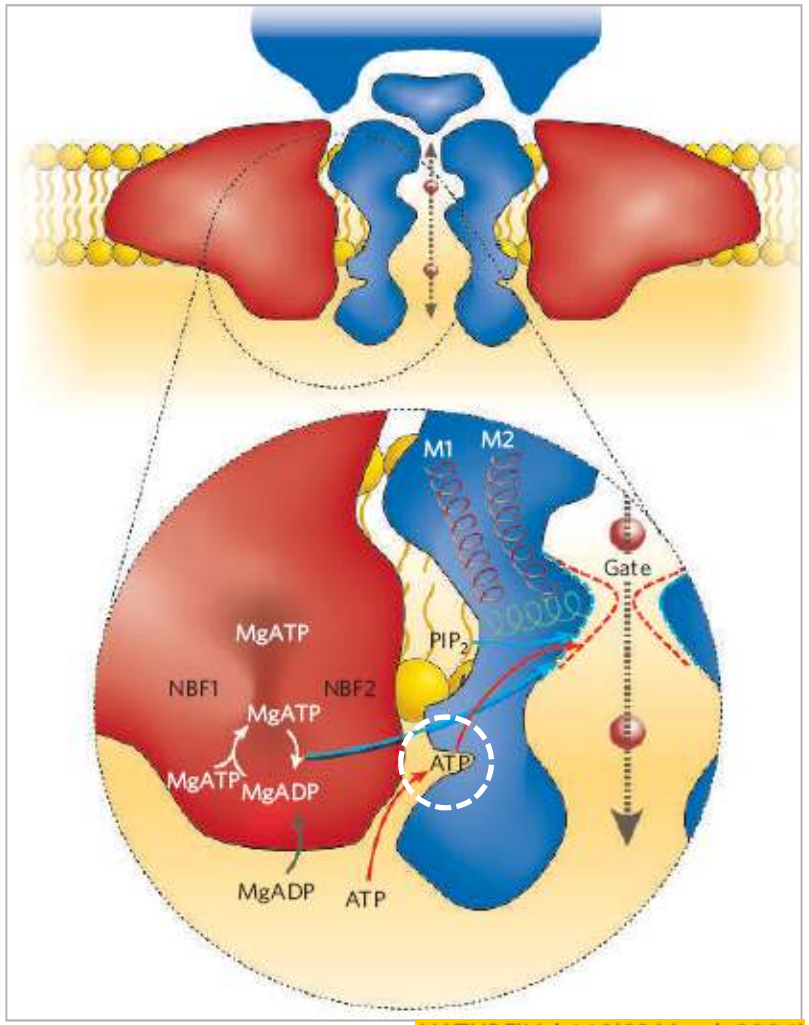


Open probability(P_O): high P_O low P_O

Channel opening is inhibited at high ATP/ activated at low ATP

K_{ATP} channels:
Molecular sensors of cellular metabolism; targets for anti-diabetics



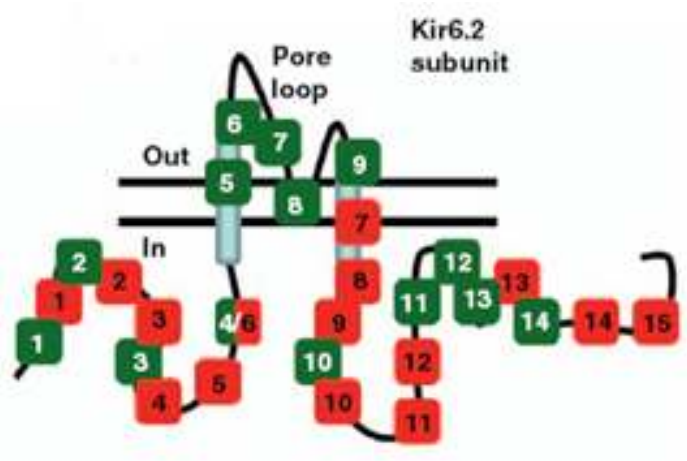


NATURE|Vol 440|23 March 2006|

- **Kir6.2**:
  ATP binding site (inhibition)
  PIP2 binding site (activation)
- **SUR1**:
  MgADP binding site (activation)
  Sulfonylurea binding site (inhibition)

K_{ATP} channel mutation causes neonatal diabetes or hyperinsulinemia

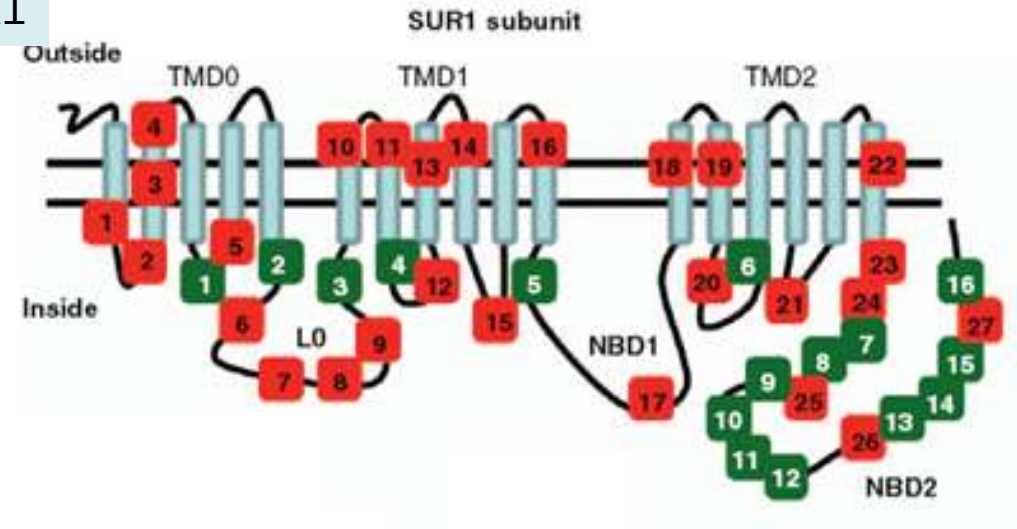
Kir6.2



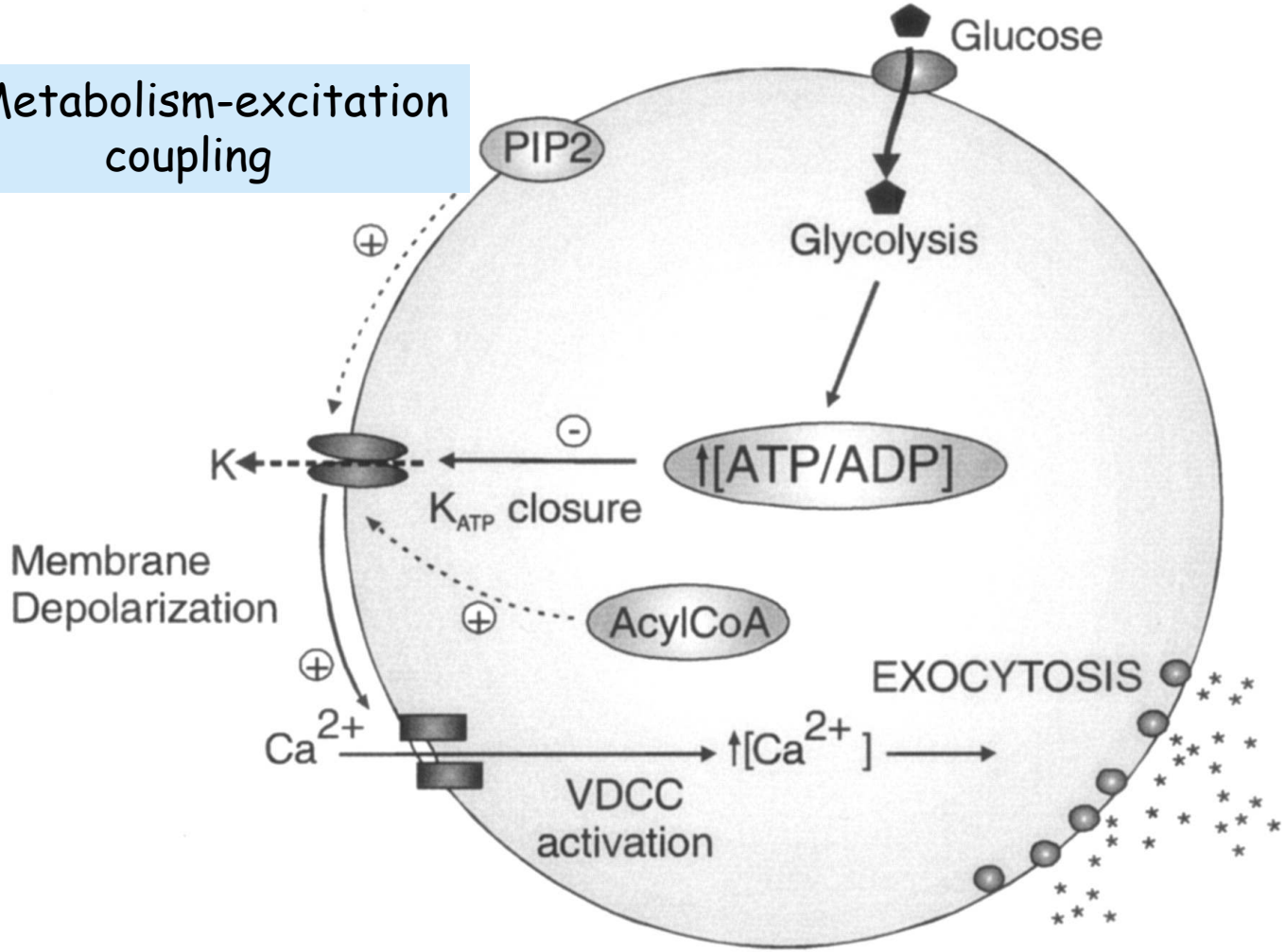
Gain of function → neonatal diabetes

Loss of function → hyperinsulinemia

SUR1



The role of pancreatic KATP channel in insulin secretion.



Koster J C et al. Diabetes 2005;54:3065-3072

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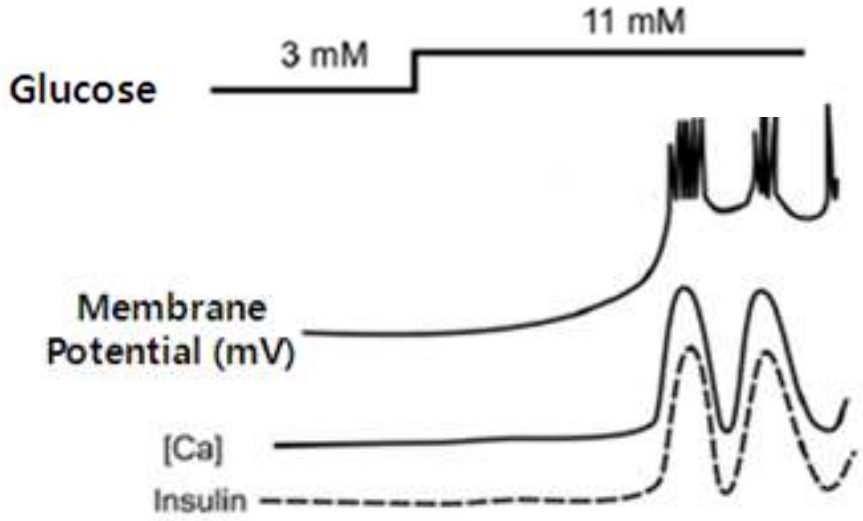

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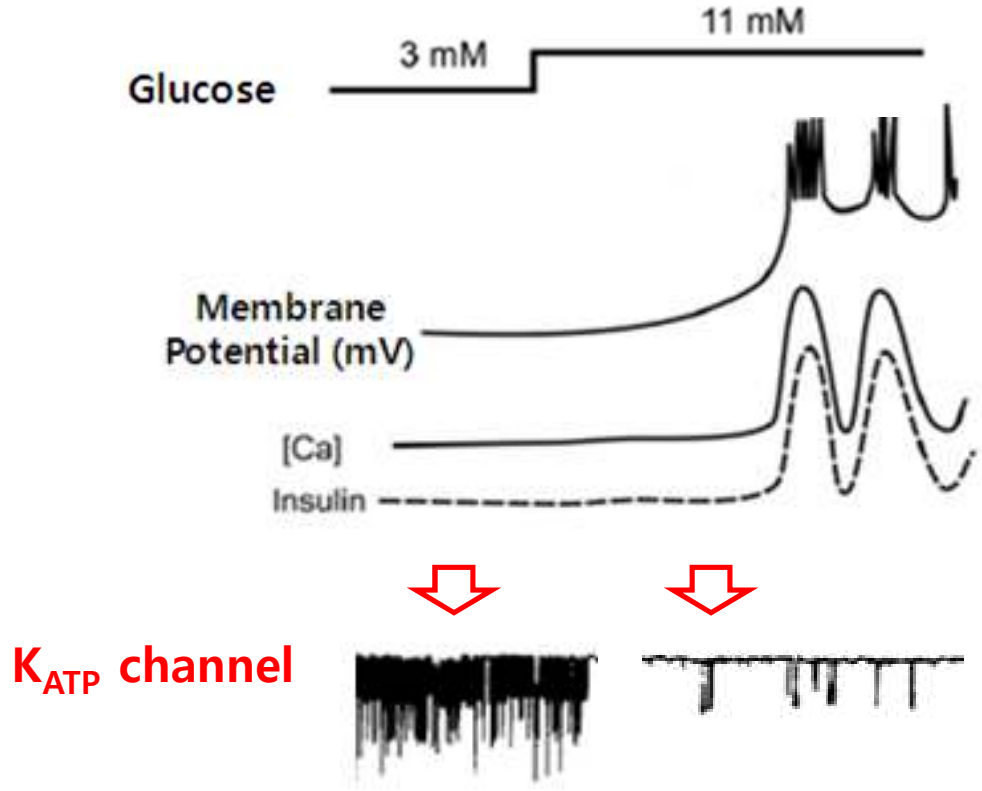
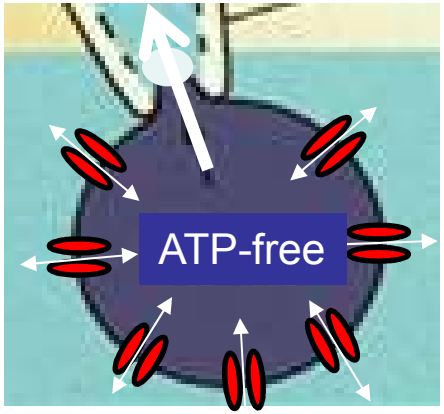


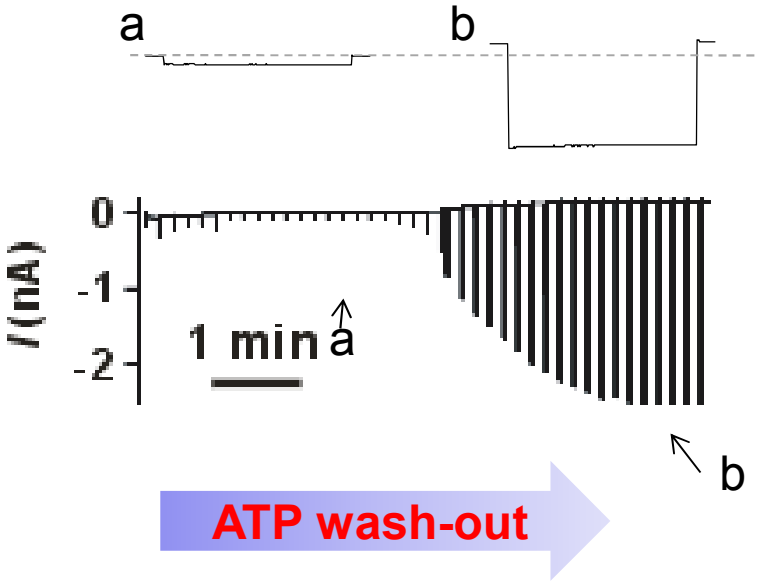
open $\xrightarrow{ATP}$ close

"Glucose induces K_{ATP} channel closure, leading to depolarization"

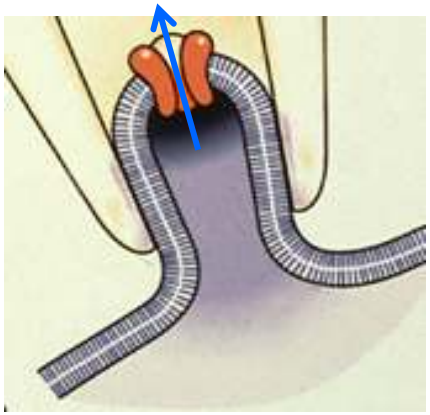
Methods for recording K_{ATP} channels

- Whole-cell recording

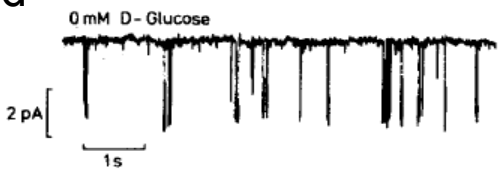




- Single channel recording



Cell-attached



Excision of patch

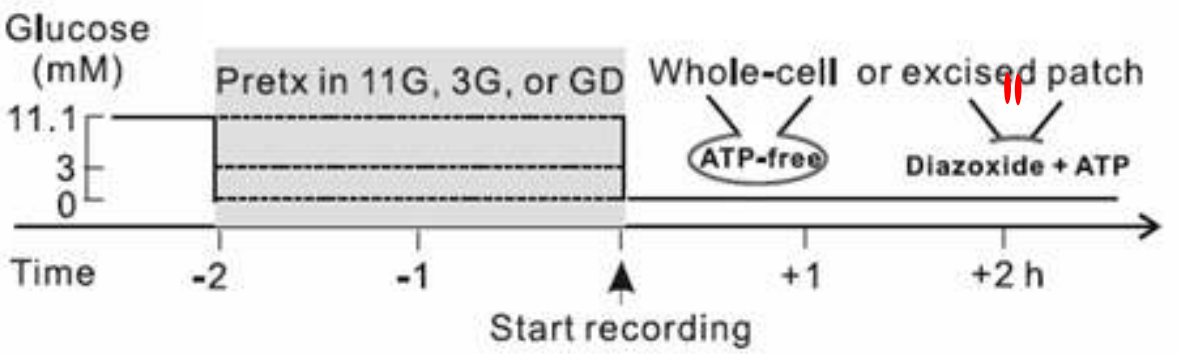
Inside-out

Unexpected observation:

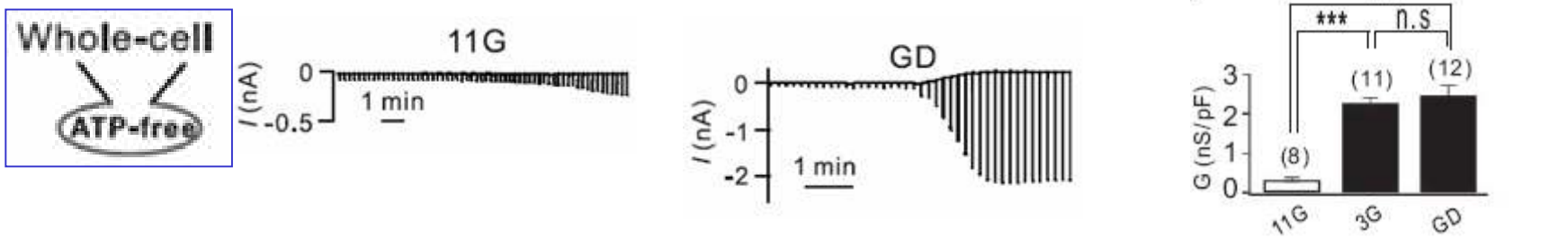
Glucose deprivation increases K_{ATP} currents in ATP-free conditions

A

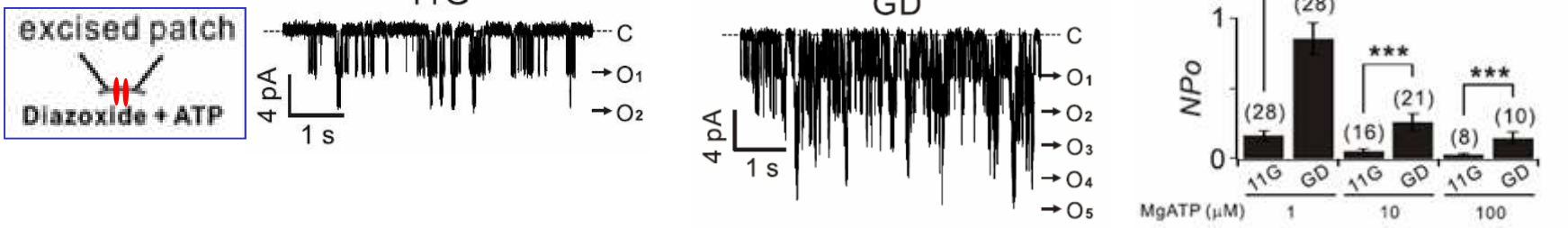
Rat pancreatic β-cells or INS-1 cells



B

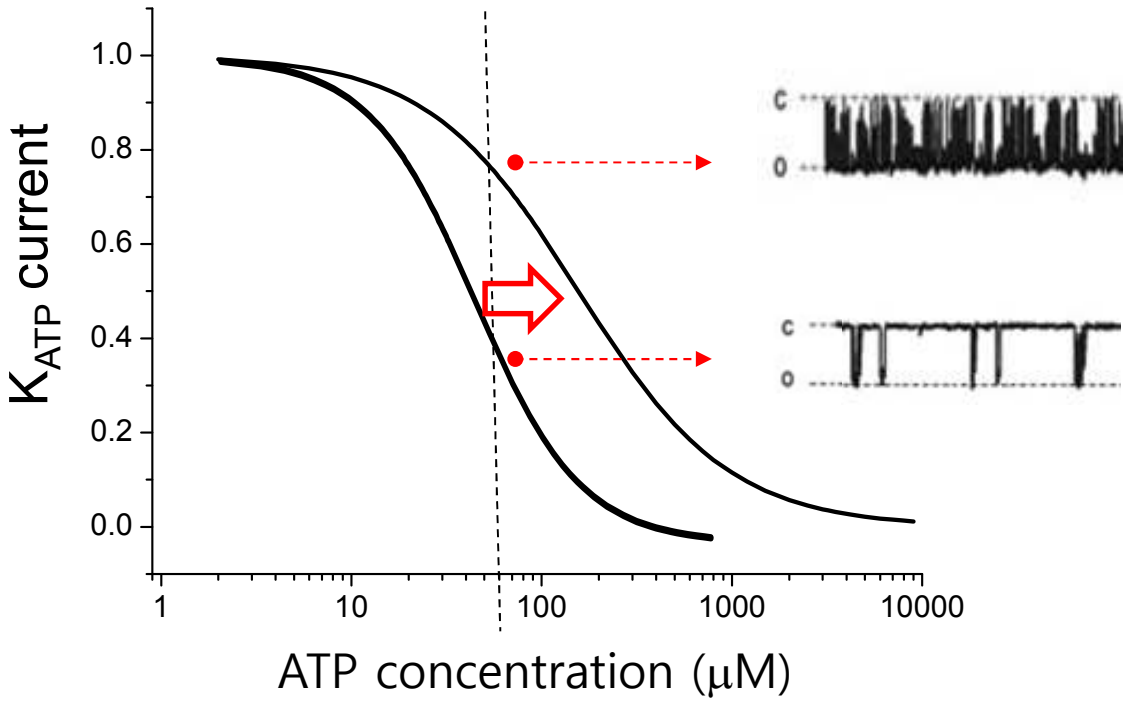


C



K_{ATP} current regulation by changing ATP-sensitivity

⇨ Decrease in ATP sensitivity by MgADP, PIP_2

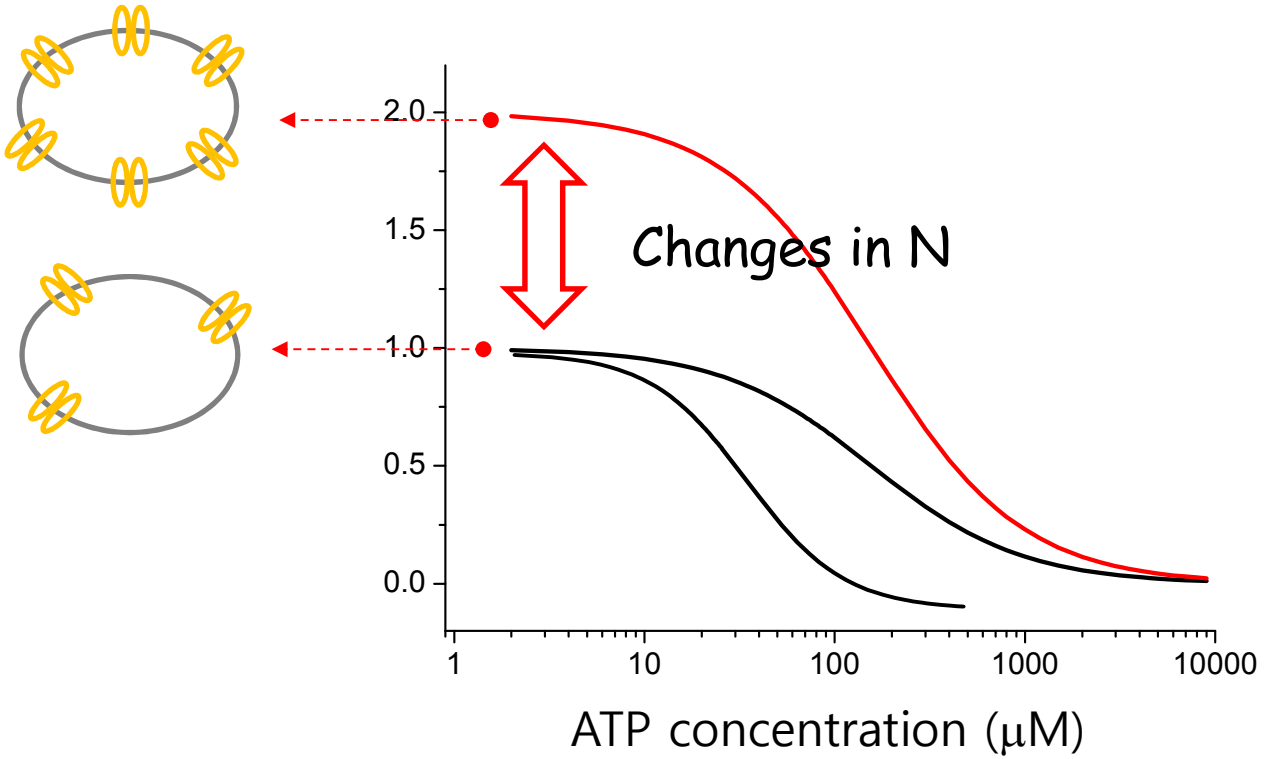


high P_O
at low sensitivity

low P_O
at high sensitivity

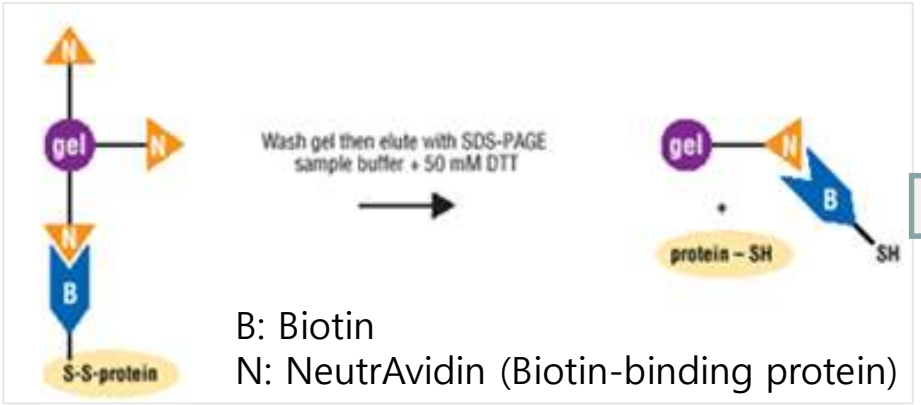
K_{ATP} current changes in ATP-free represent changes in channel density

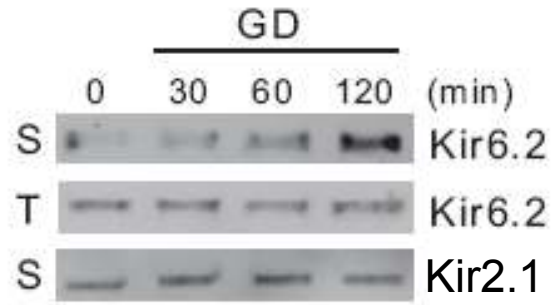
K_{ATP} current = N (channel density) x P_o (open probability)



K_{ATP} channel surface density was increased by glucose deprivation

1. Western blot for Cell Surface Proteins (Biotinylation technique)

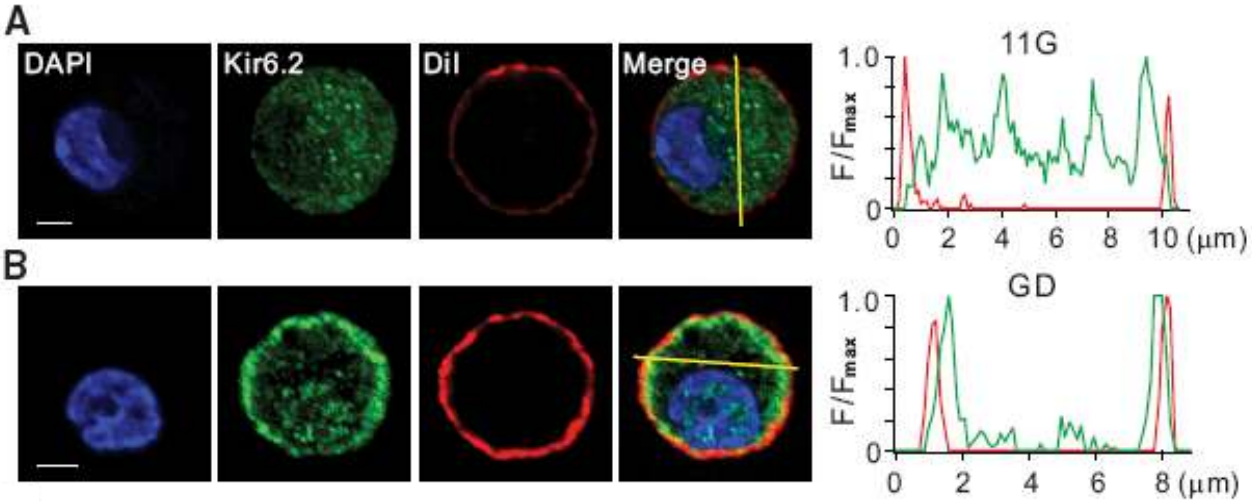




2. Immunocytochemistry

11 mM Glucose

GD: 2 hr in Glucose-free



Paradigm shift from gating regulation to trafficking

기존연구

low P_O

Gating mechanism

high P_O

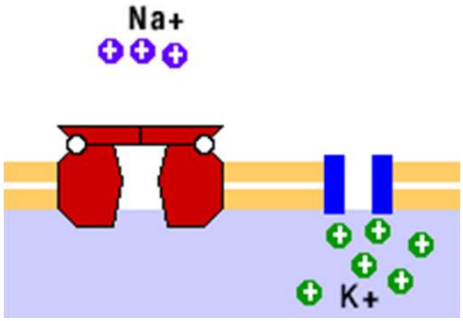
본연구

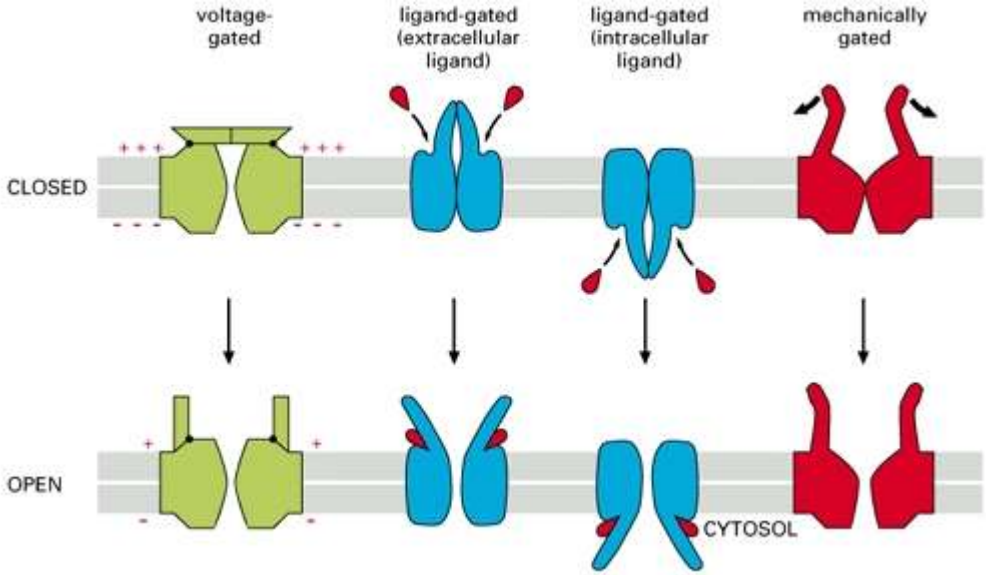
low N

Trafficking mechanism

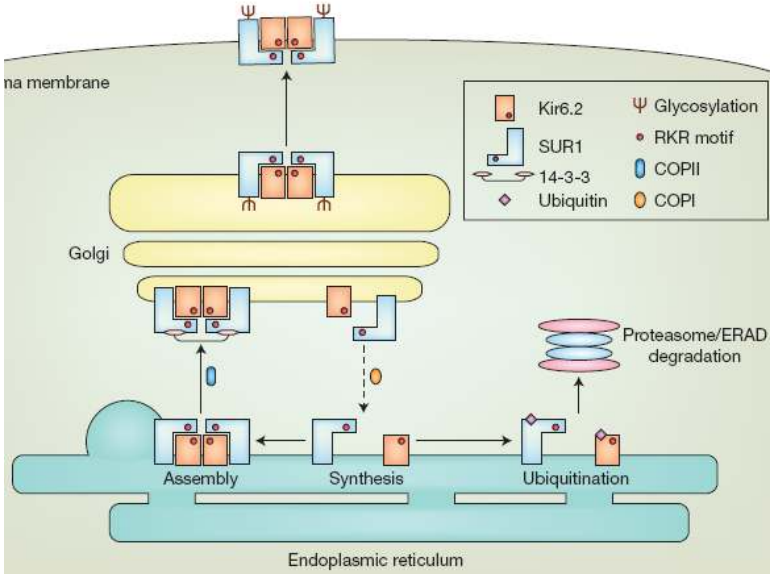
high N

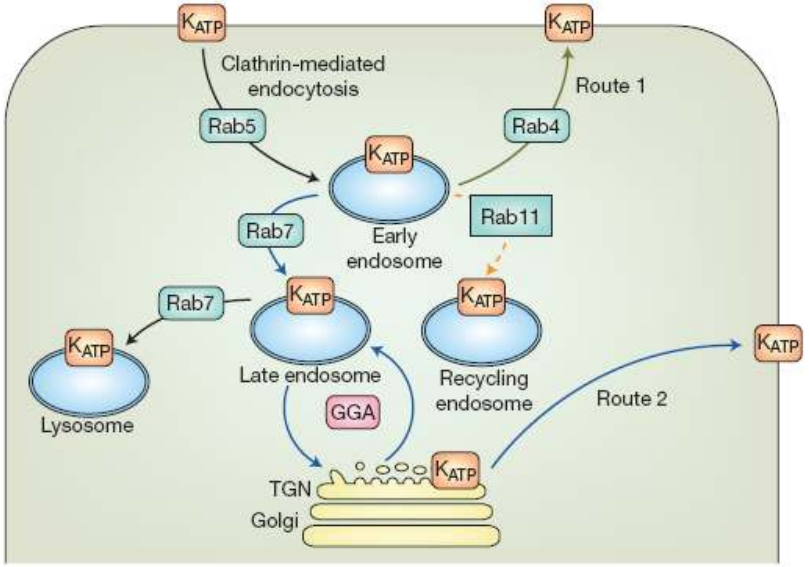
Gating mechanism



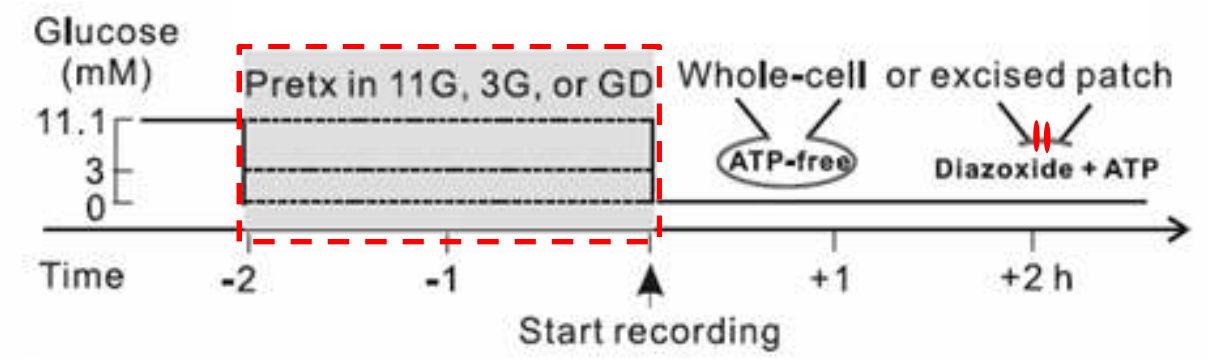


Trafficking mechanism



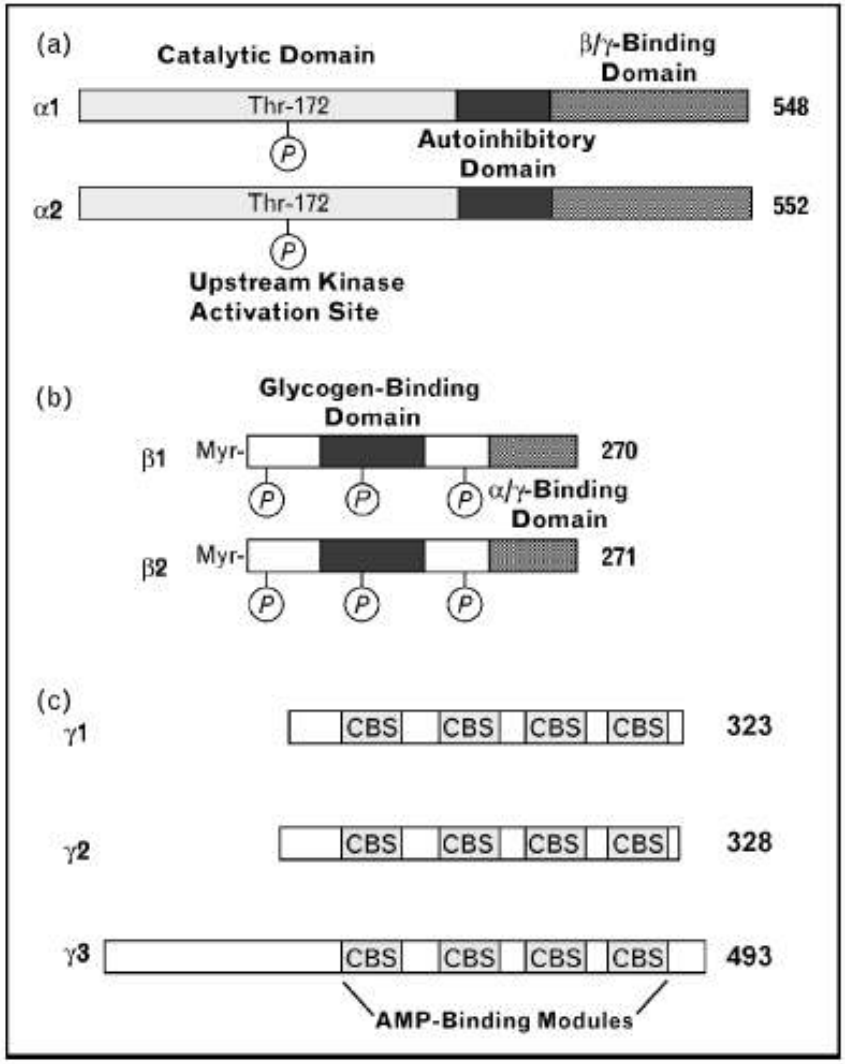


Question 1

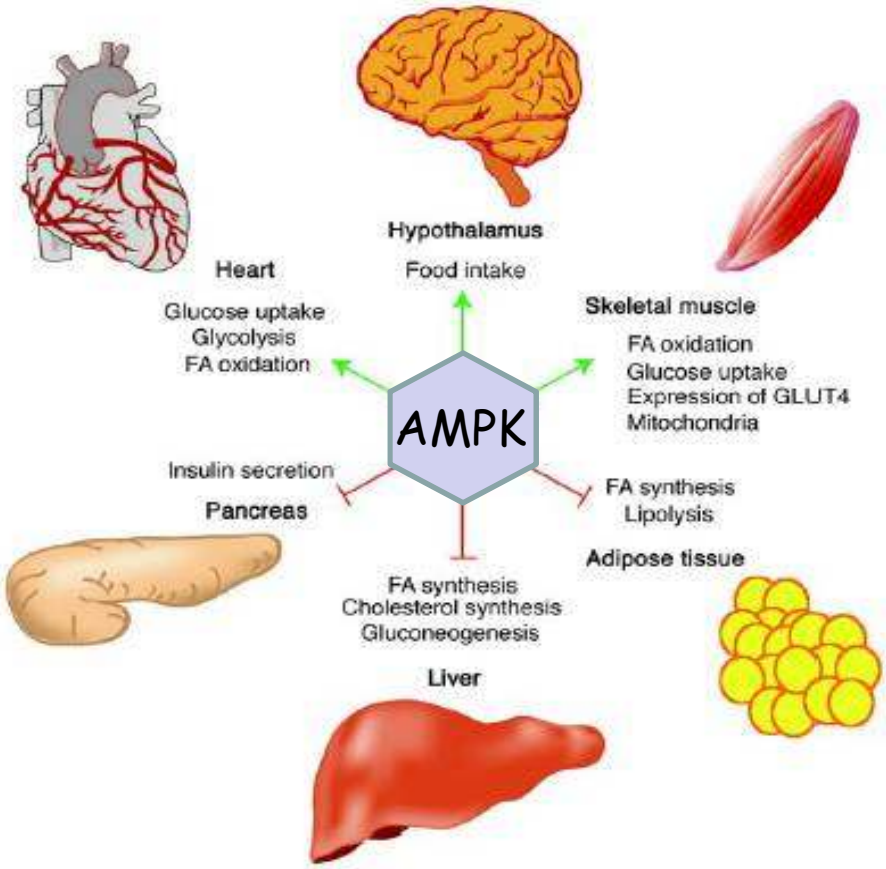


Which signaling mechanism activated during glucose deprivation promotes KATP channel trafficking?

AMPK, AMP-activated protein kinase, a sensor of cellular energy status

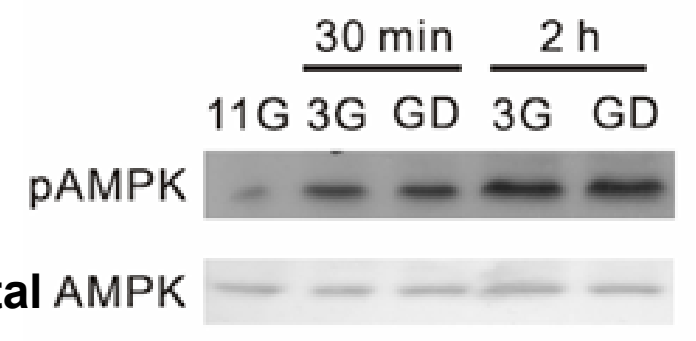


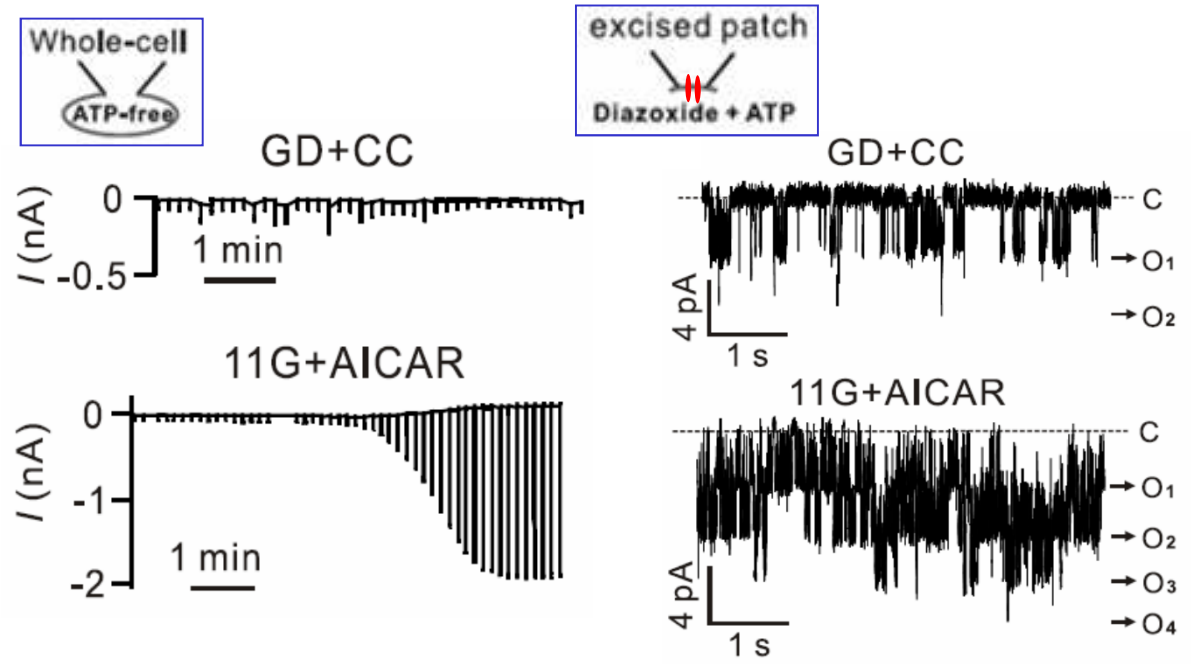
Roles of AMPK



From Current Opinion in Nephrology and Hypertension 2005

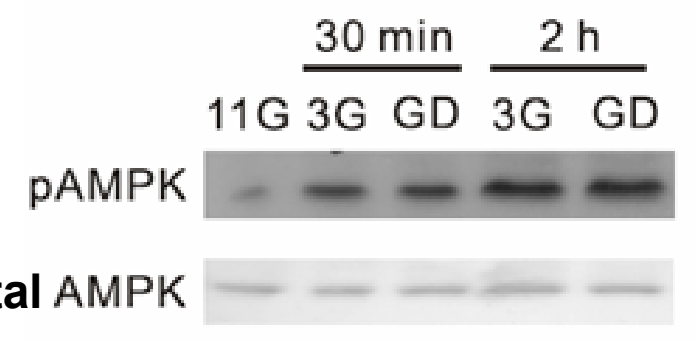
AMPK is involved in increases in K_{ATP} conductance induced by glucose deprivation

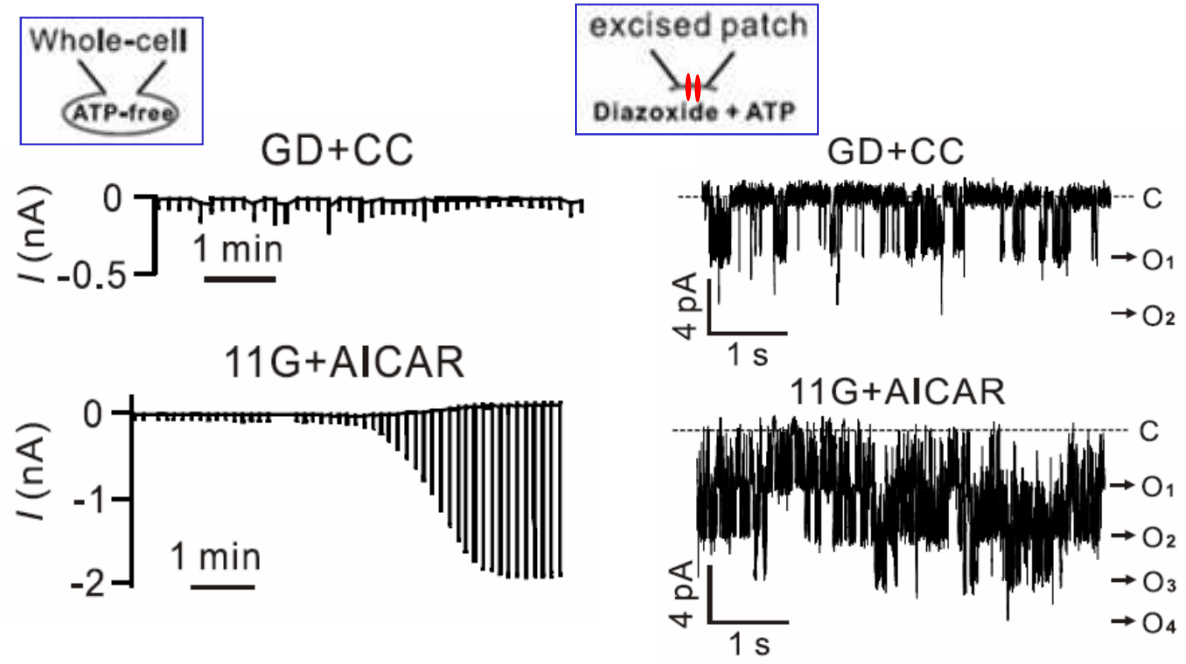


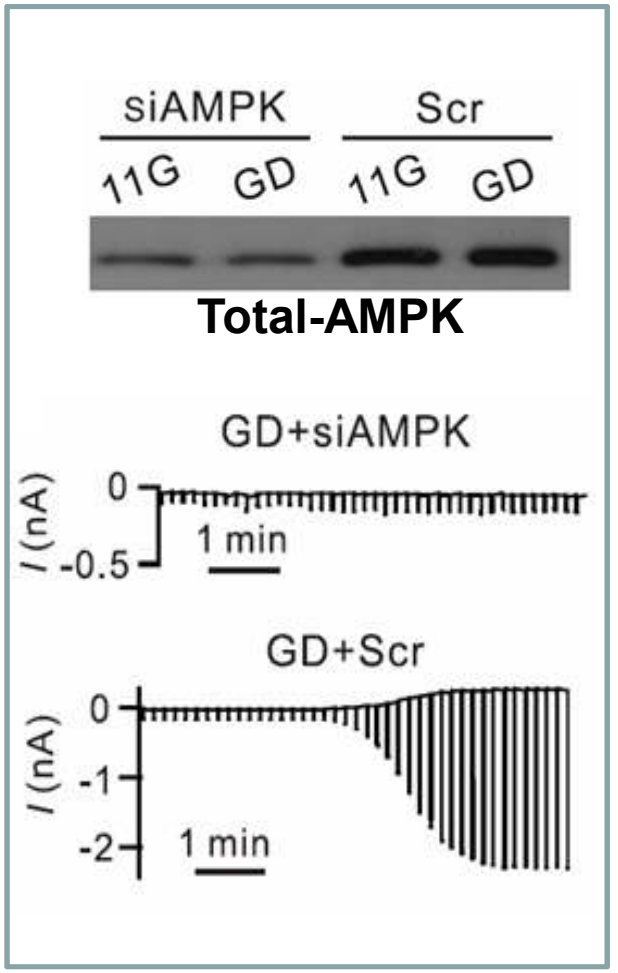


- CC : compound C, AMPK inhibitor, 10 μM
- AICAR : AMPK activator, 0.5 mM

AMPK is involved in increases in K_{ATP} conductance induced by glucose deprivation



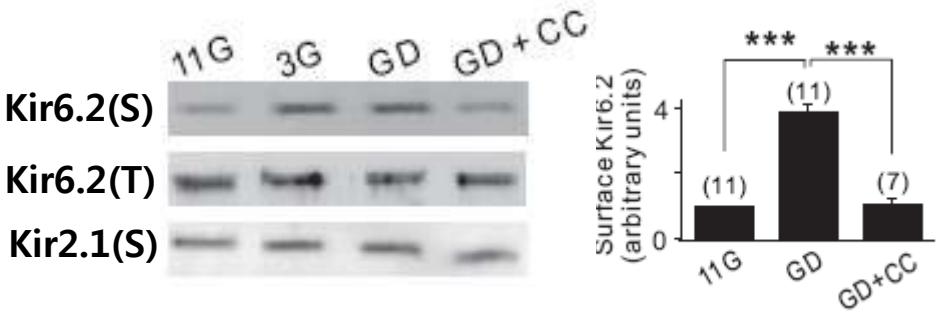




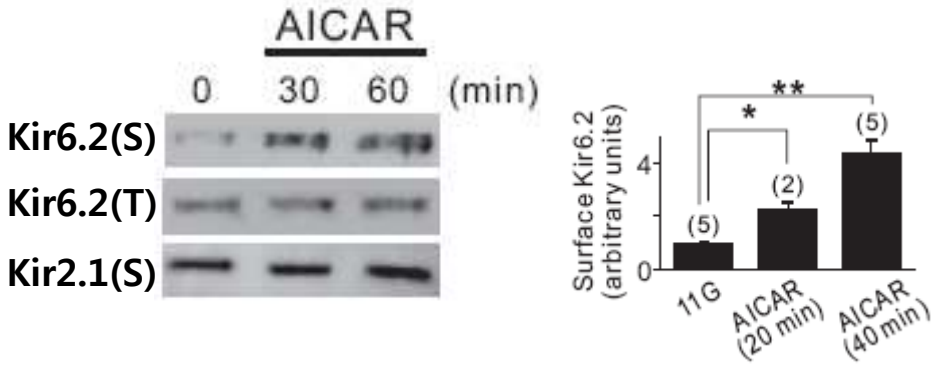
- CC : compound C, AMPK inhibitor, 10 μM
- AICAR : AMPK activator, 0.5 mM

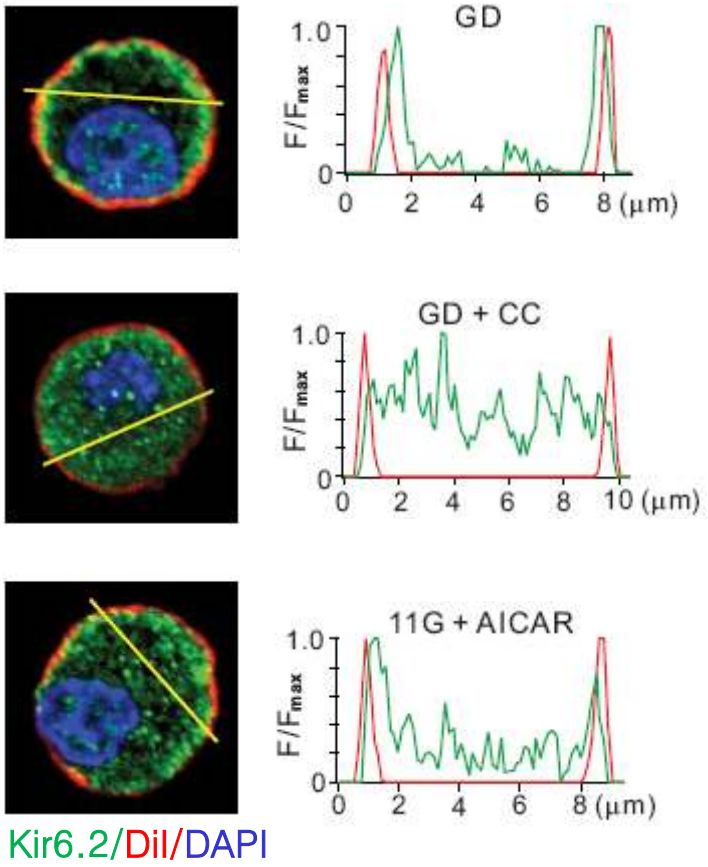
K_{ATP} channel surface density was increased by AMPK activation, and inhibited by AMPK inhibitor

1. Effect of Compound C, AMPK inhibitor

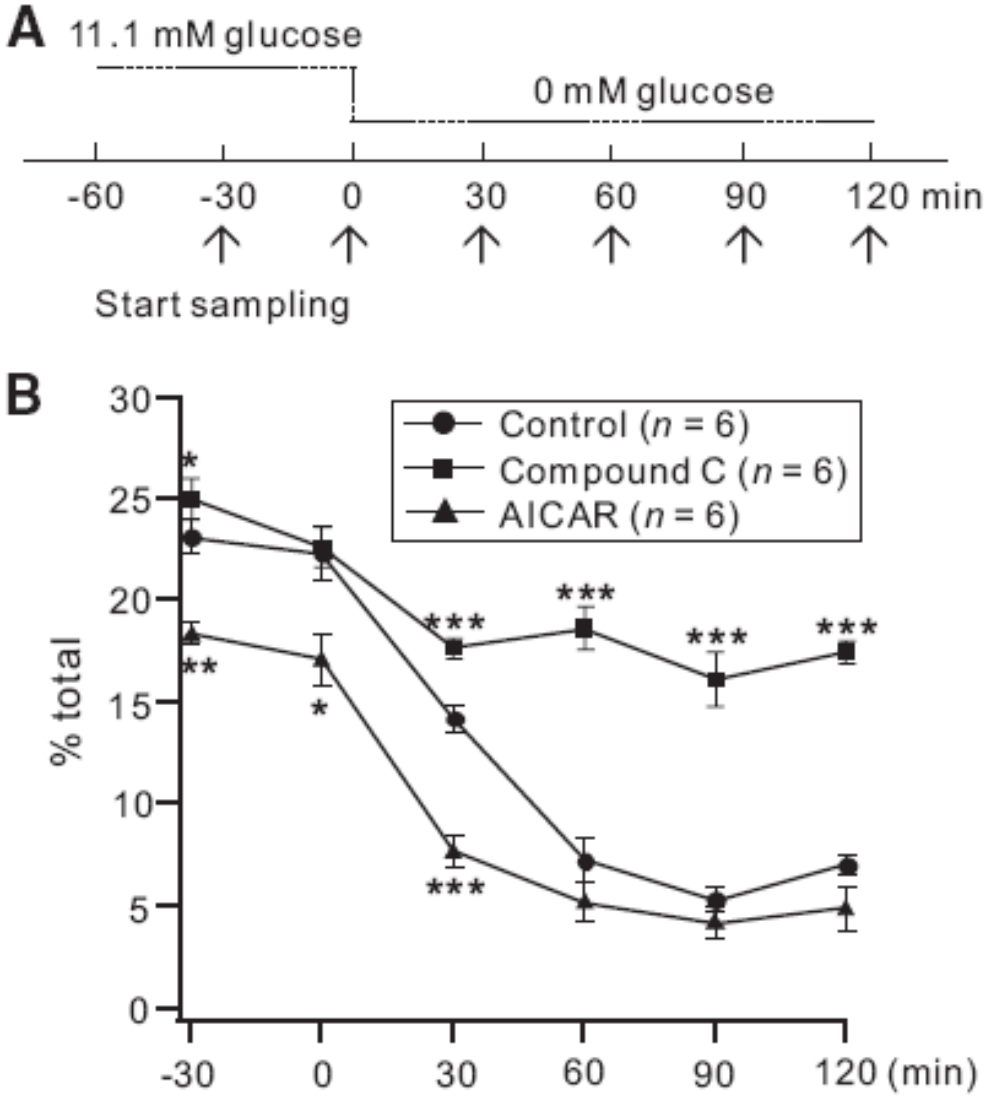


2. Effect of AICAR, AMPK activator





Suppression of insulin secretion by glucose deprivation requires AMPK signaling

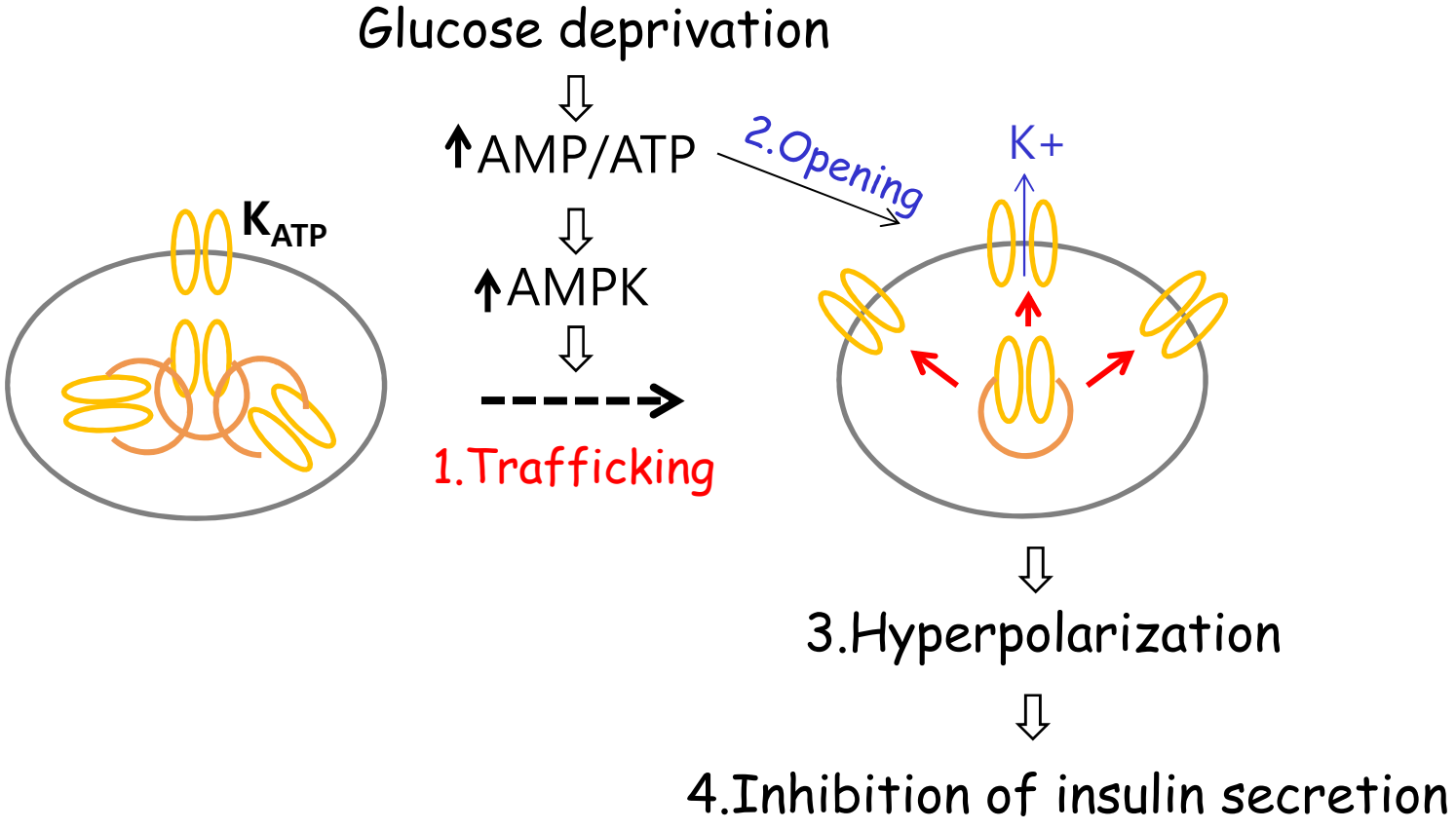


계명대 송대규 교수 Lab

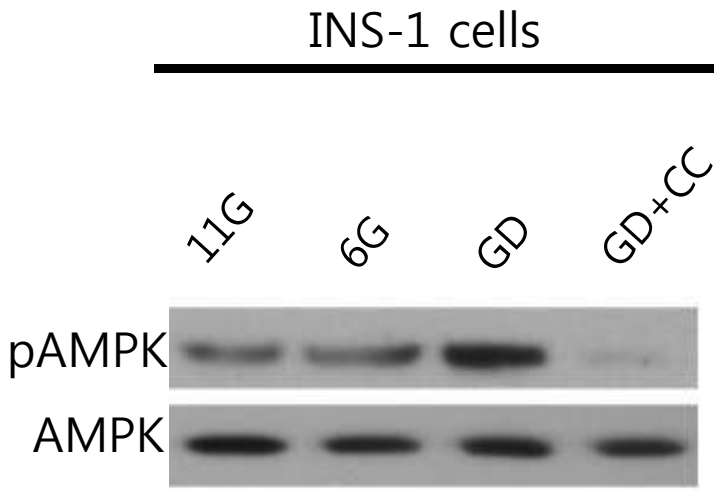
ORIGINAL ARTICLE

Glucose Deprivation Regulates K_{ATP} Channel Trafficking via AMP-Activated Protein Kinase in Pancreatic β-Cells

Ajin Lim,[1,2] Sun-Hyun Park,[1,2] Jong-Woo Sohn,[1,2] Ju-Hong Jeon,[2] Jae-Hyung Park,[3] Dae-Kyu Song,[3] Suk-Ho Lee,[1,2] and Won-Kyung Ho[1,2]

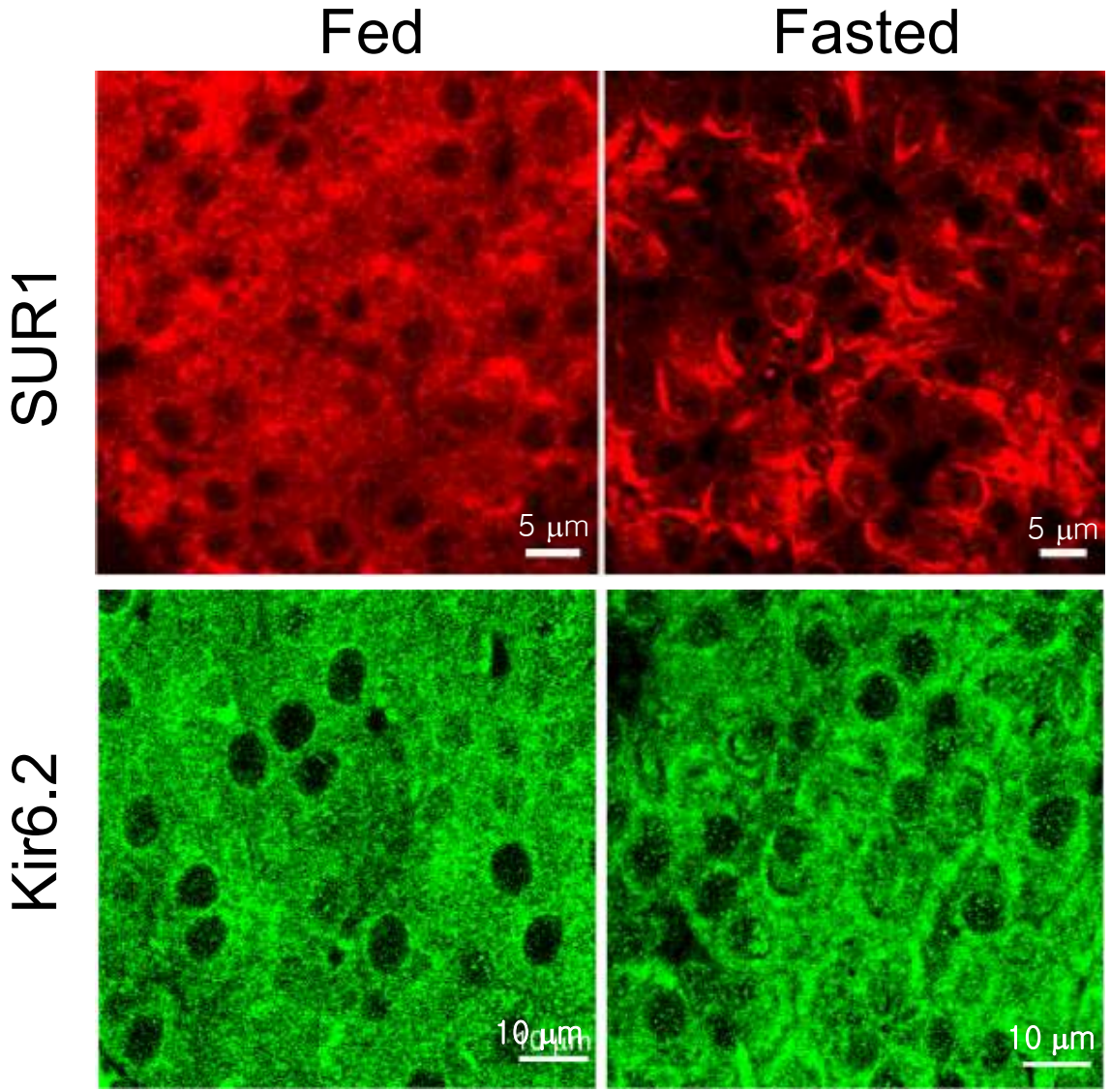


Physiological significance is uncertain:
AMPK was not activated at fasting glucose level in INS-1 cells



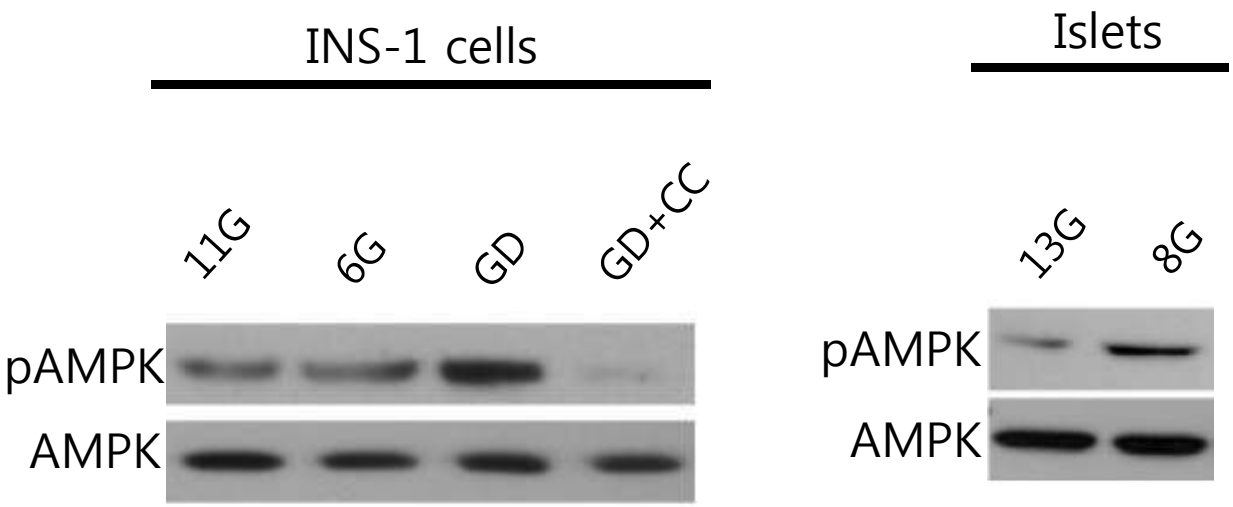
Question 2

- Does AMPK-dependent trafficking occur *in-vivo* during fasting?



Glucose: 244 mg/dl (13.5 mM) 138 mg/dl (7.7 mM)

AMPK is activated at fasting glucose not *in vitro*, but *in vivo*



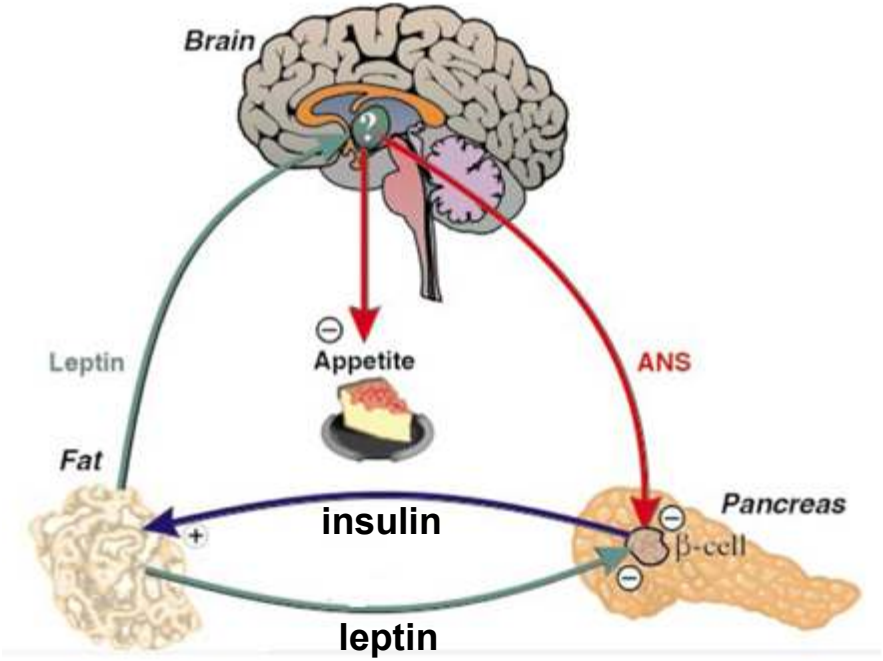
Hypothesis:
Endogenous ligands promote AMPK activation in normal fasting state

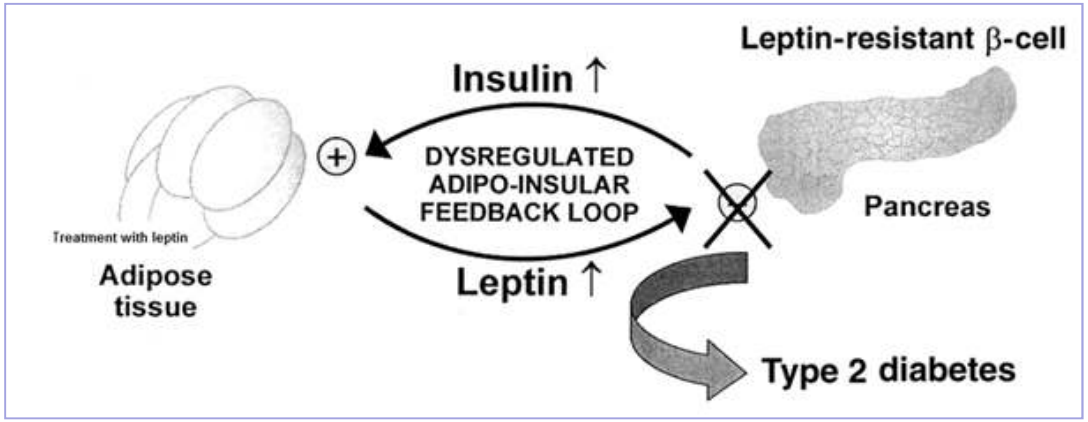
Leptin

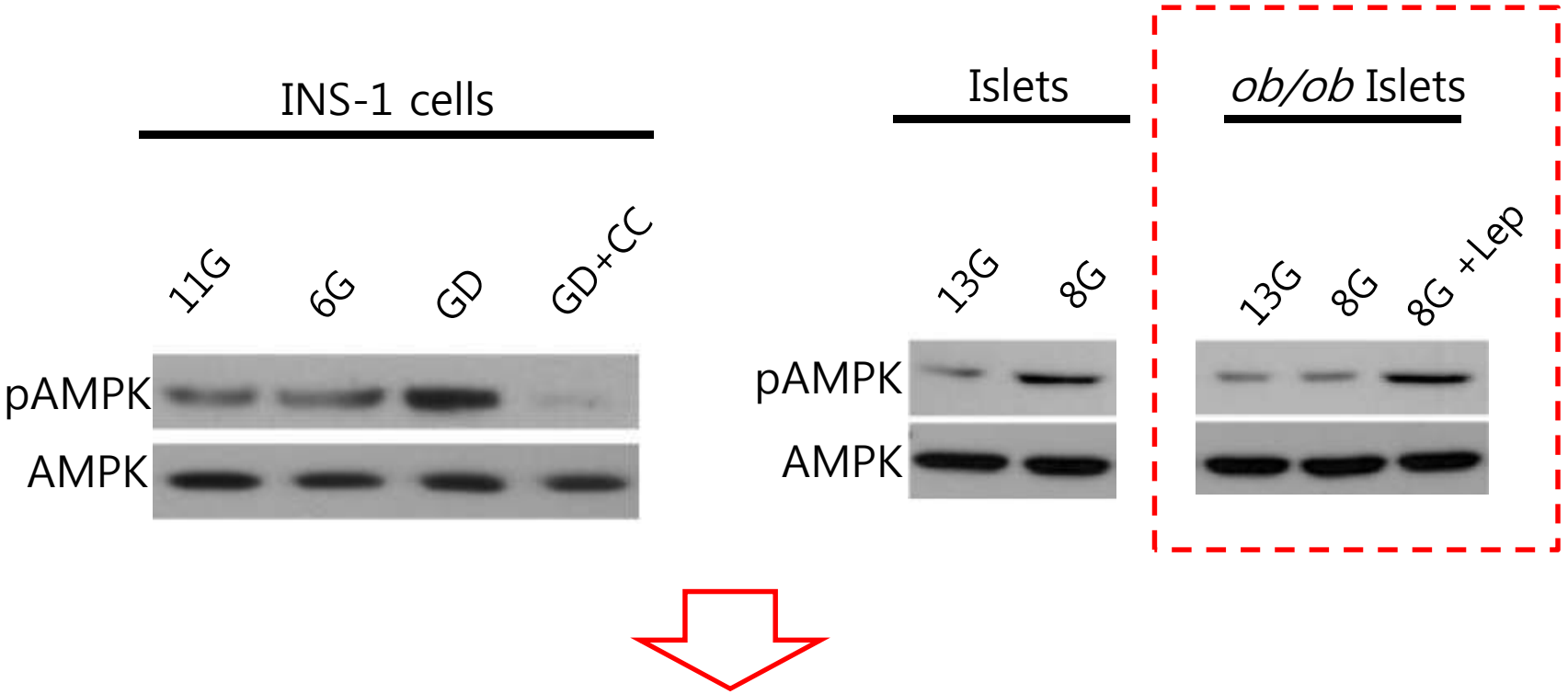
a pivotal regulator of energy homeostasis

***ob/ob* mouse**

Leptin Deficient *ob/ob* mice show
-hyperinsulinemia
-diabetes

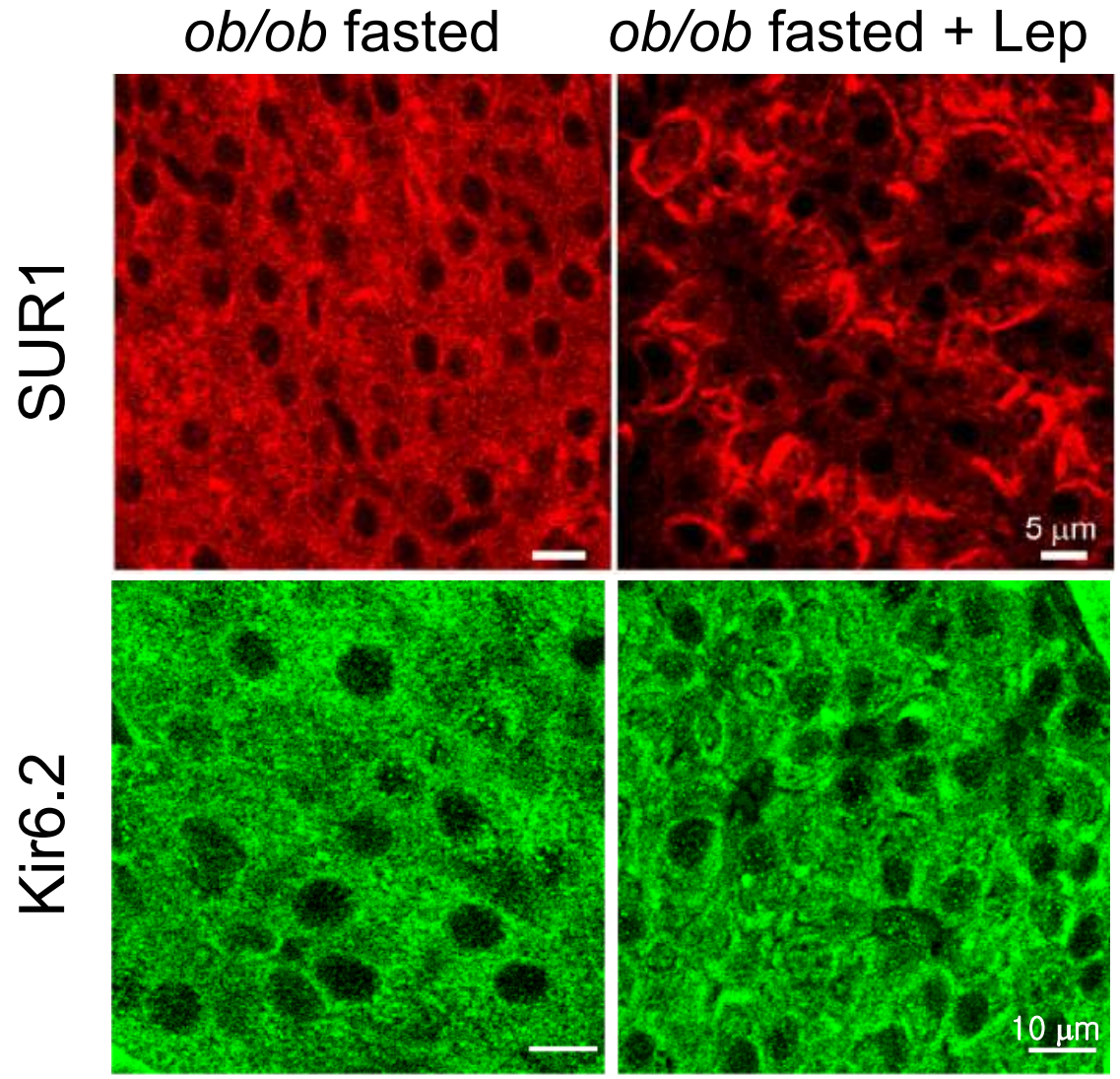






AMPK can be activated in normal fasting state in the presence of leptin

K_{ATP} channel trafficking is impaired in leptin deficiency

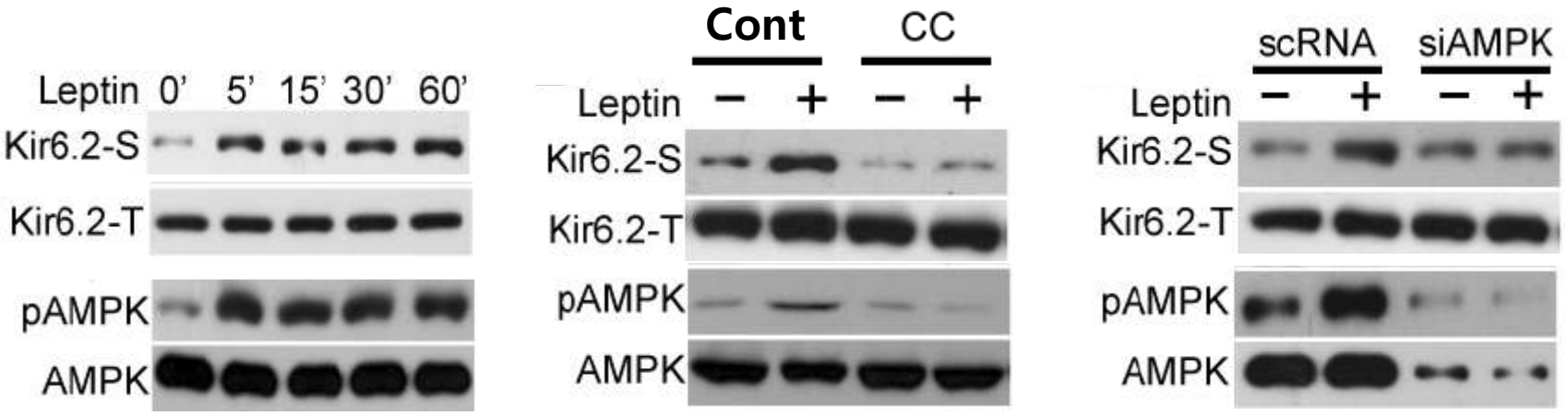


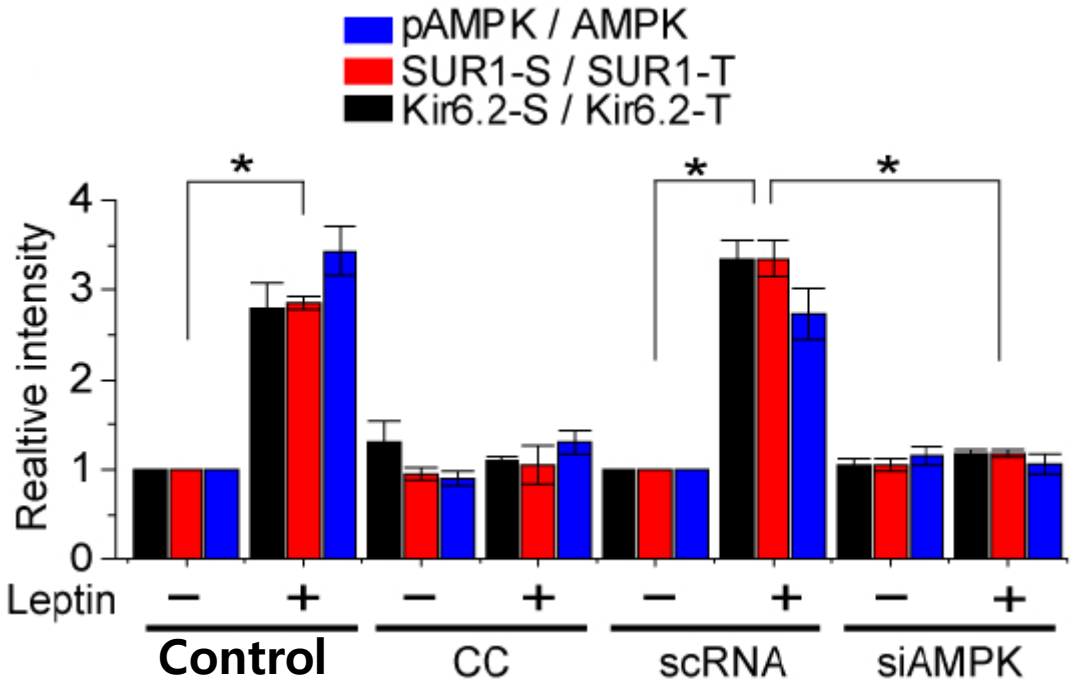
Glucose: 252 mg/dl 152 mg/dl

Question 3

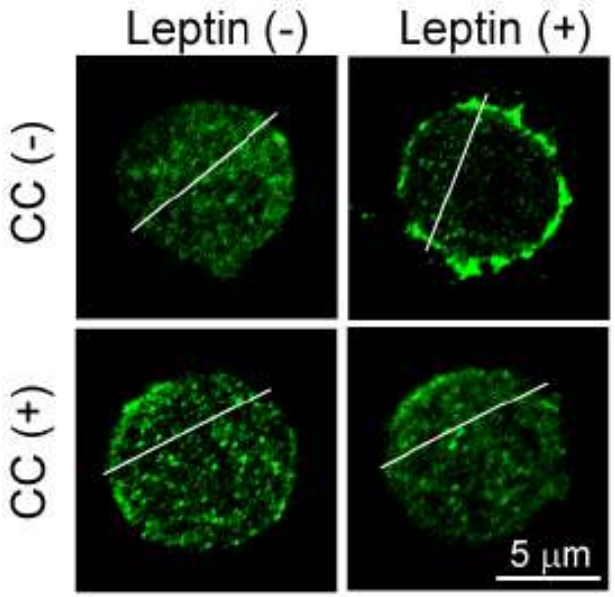
- Mechanism of leptin-induced AMPK activation and K_{ATP} channel trafficking?

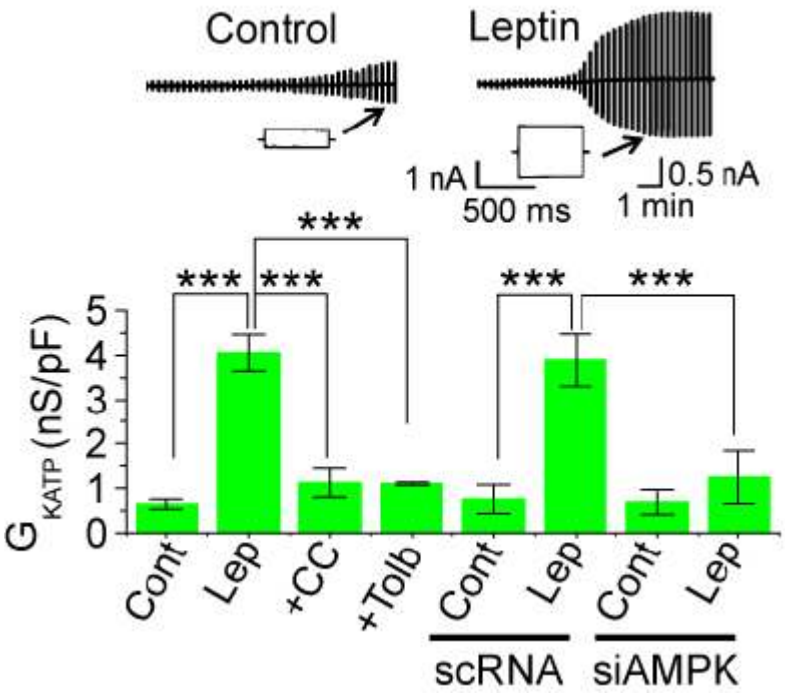
Leptin promotes AMPK activation and K_{ATP} channel trafficking



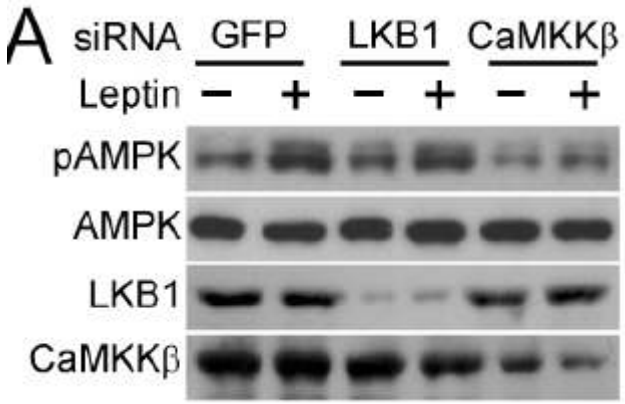


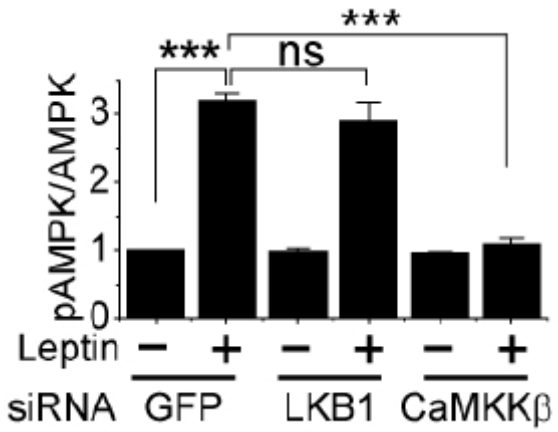
Leptin promotes AMPK activation and K_{ATP} channel trafficking

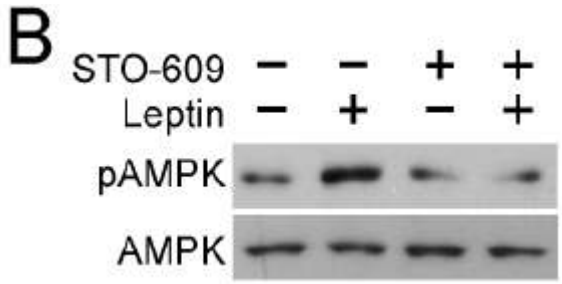


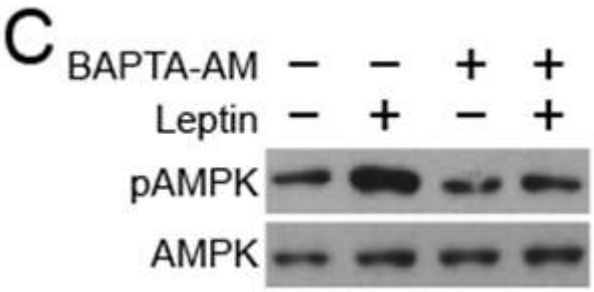


Leptin-induced AMPK activation is mediated by CaMKKβ

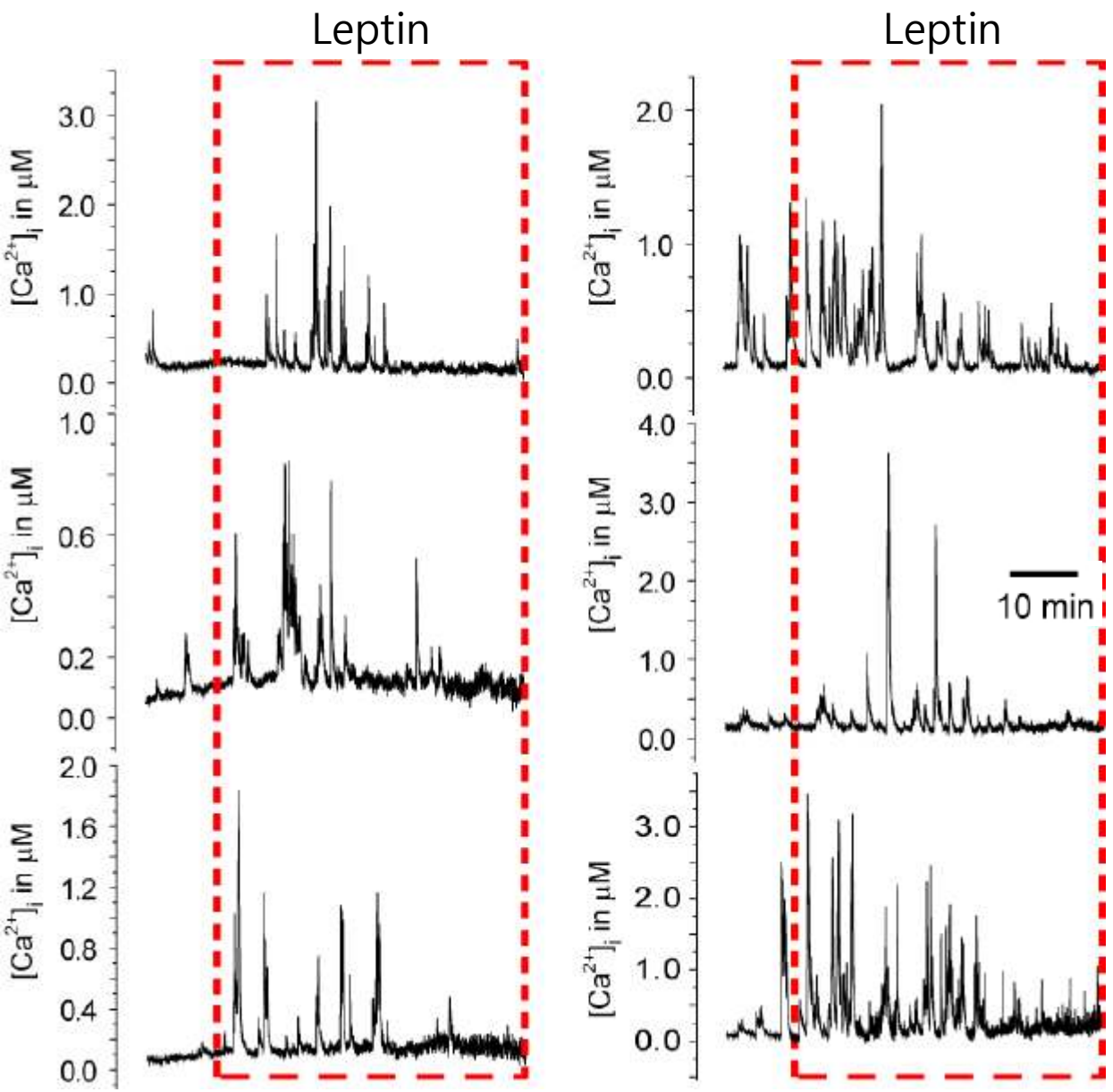




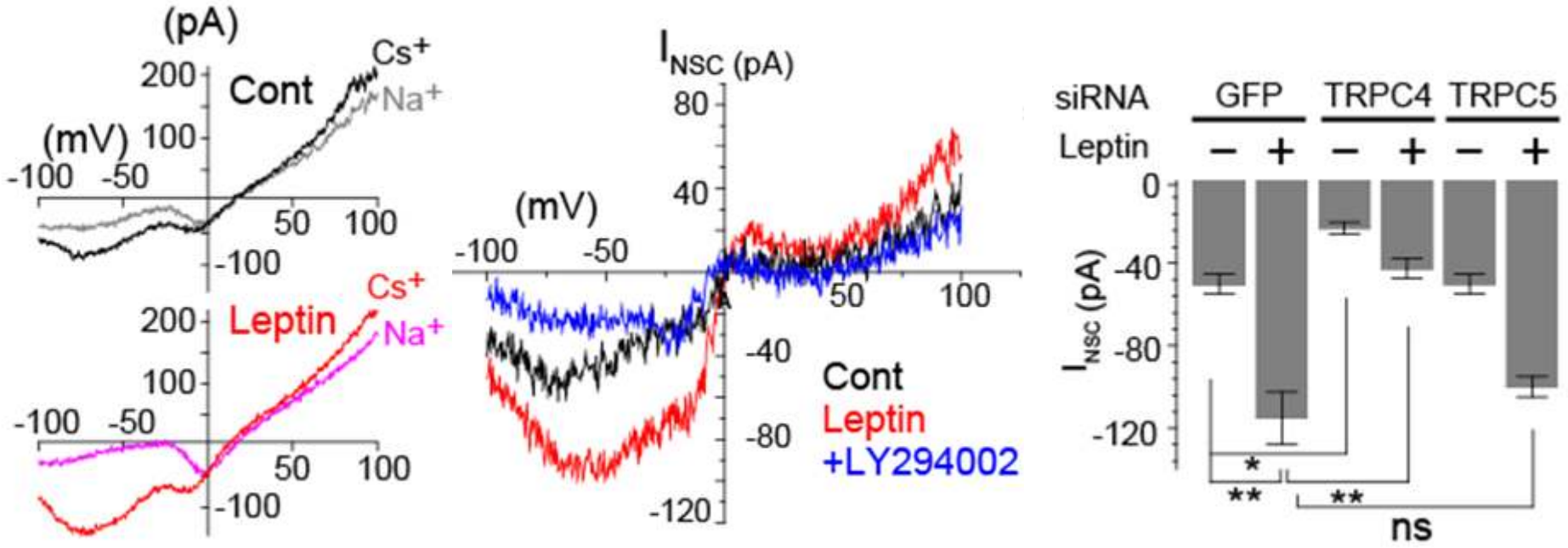




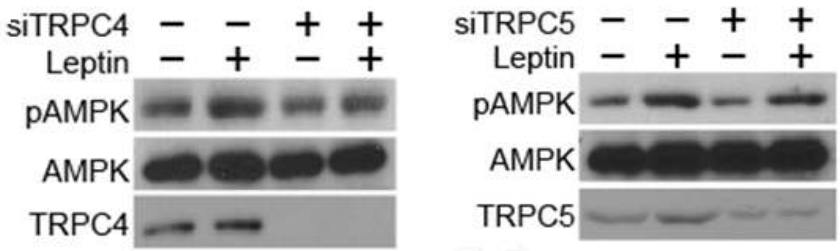
Leptin induces Ca^{2+} increases in β-cells

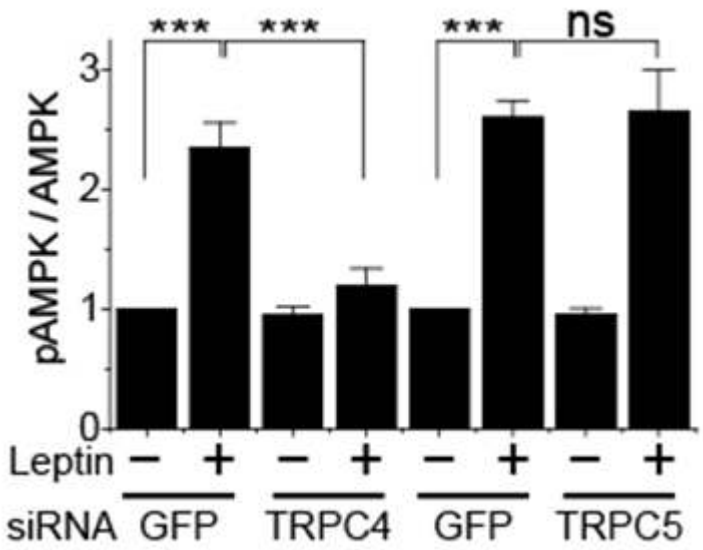


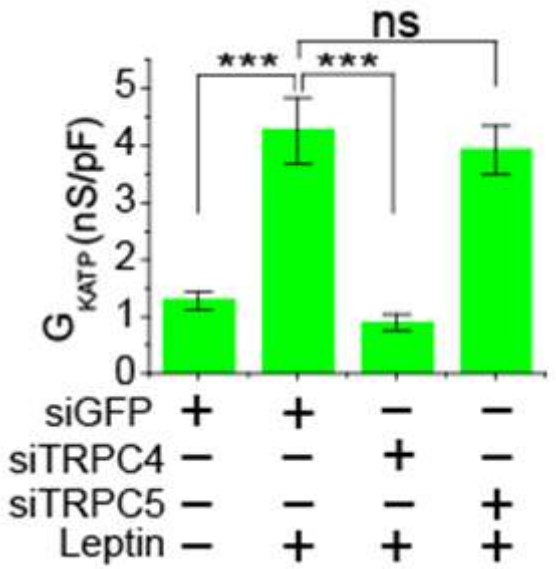
Leptin increases NSC by activating TRPC4, but not TRPC5

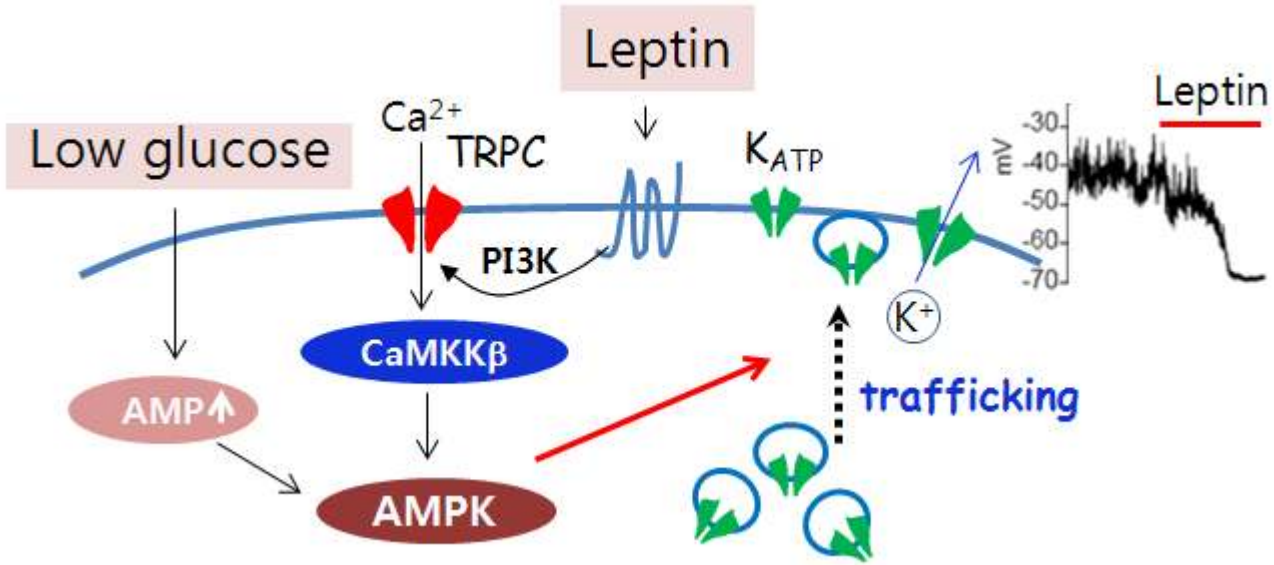


Activation of AMPK and K_{ATP} by leptin is blocked by siTRPC4





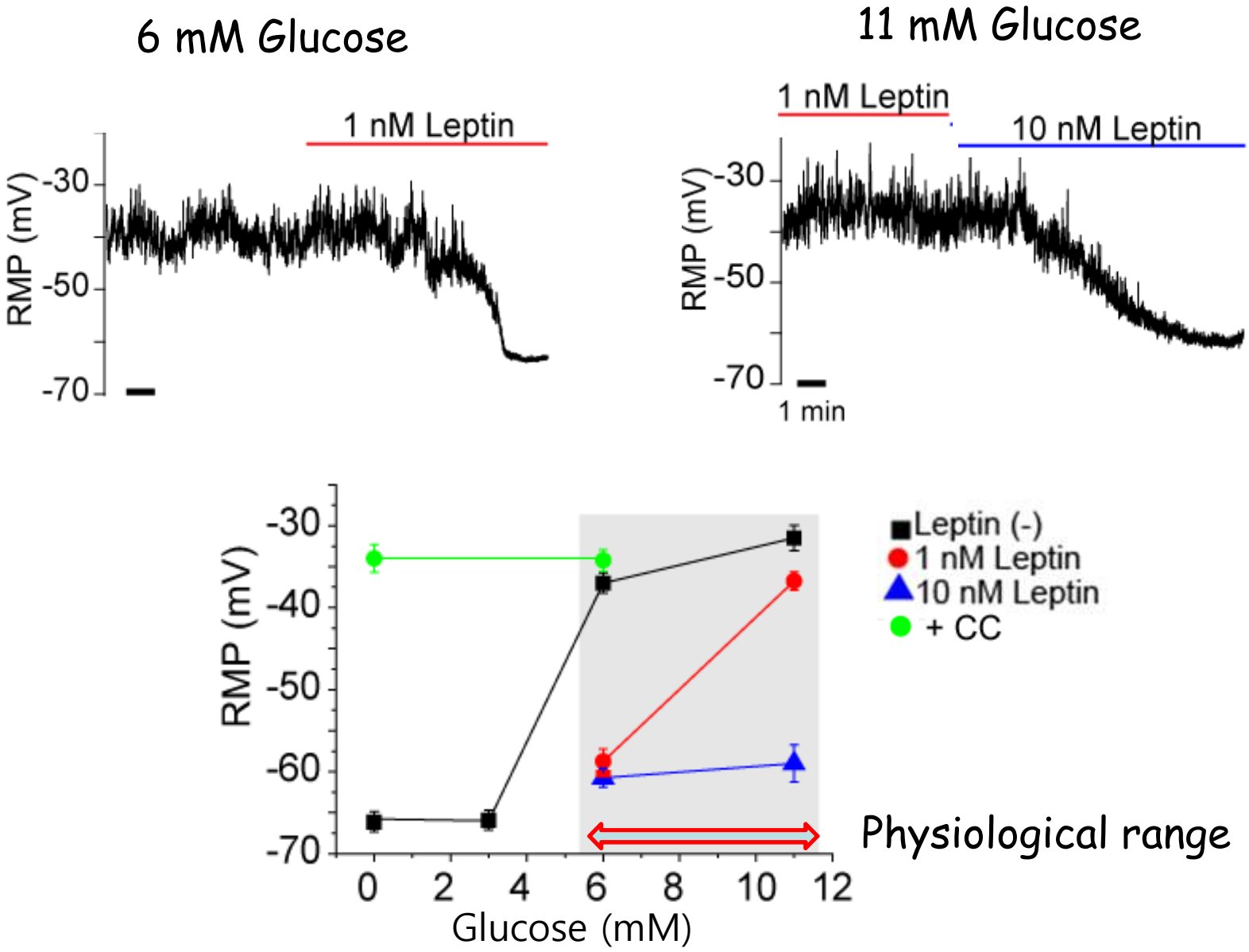



Leptin
Low glucose
Ca2+
TRPC
KATP
Leptin
-30
-40
-50
-60
-70
mV
PI3K
CaMKKβ
K+
AMP
trafficking
AMPK

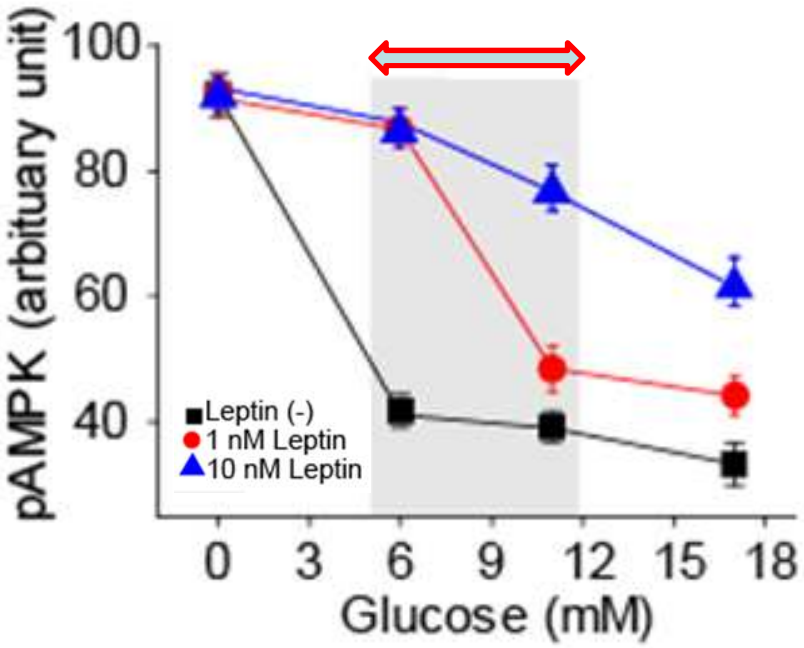
Question 4

- Functional significance of leptin in β-cell excitability

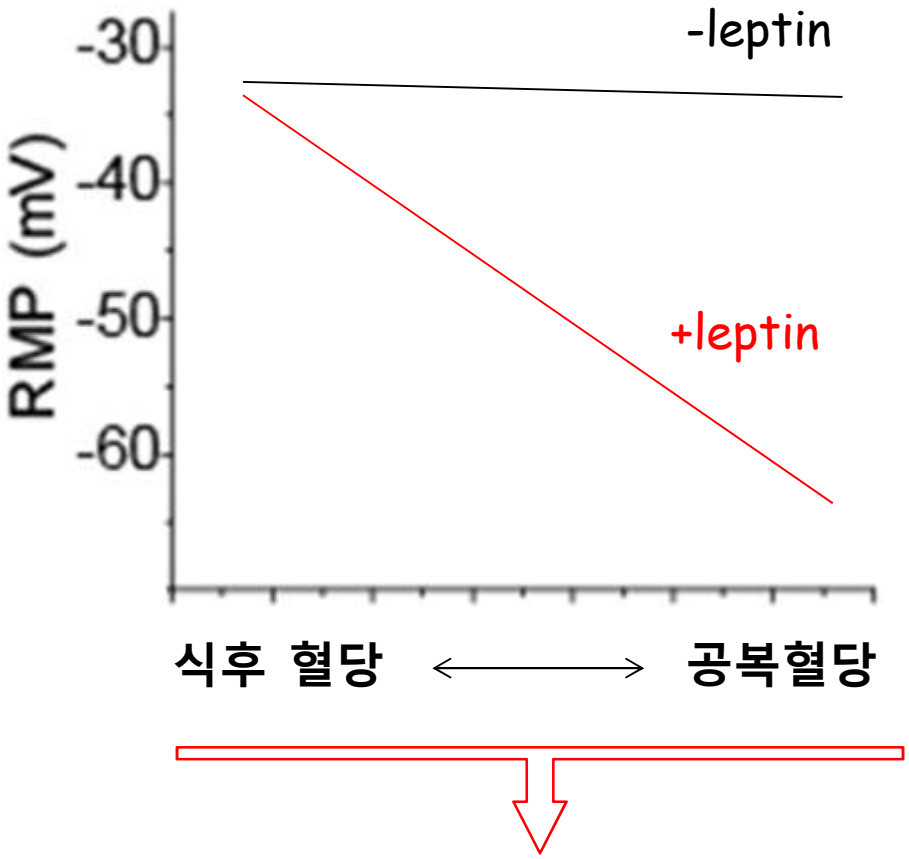
β-cell RMP responds to physiological range of glucose in the presence of low concentration of leptin



pAMPK responds to physiological range of glucose in the presence of low concentration of leptin

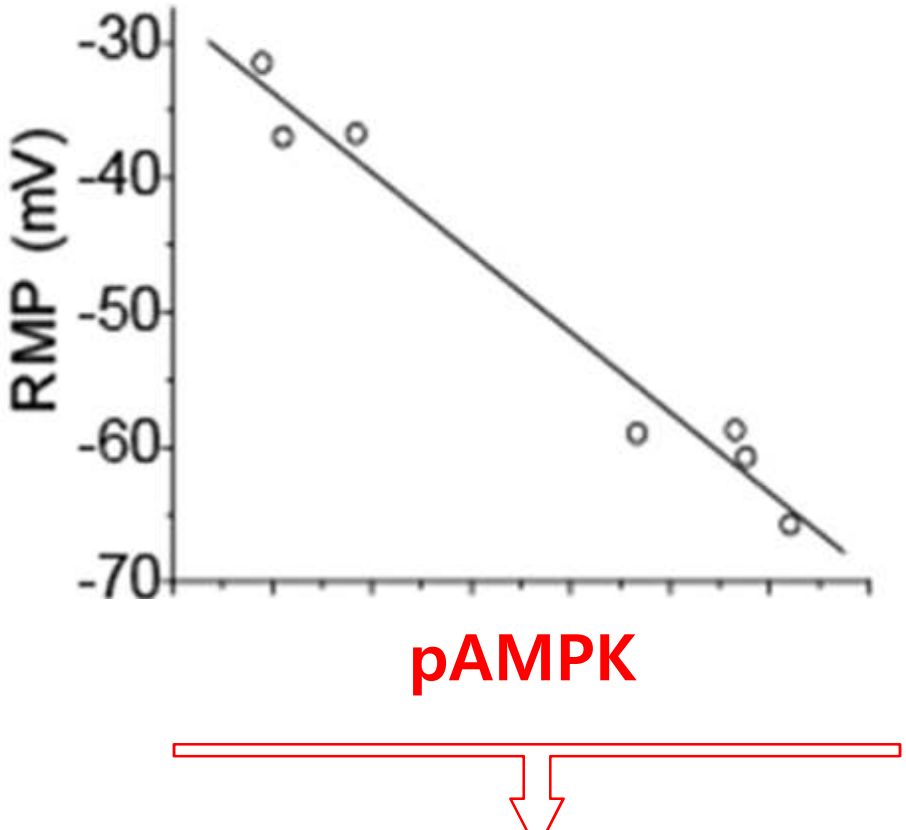


glucose (ATP_i) dependence of β-cell RMP depends on leptin



Energy status (glucose) and hormonal signal (leptin)
are integrated into AMPK activity

β-cell RMP correlates linearly with pAMPK

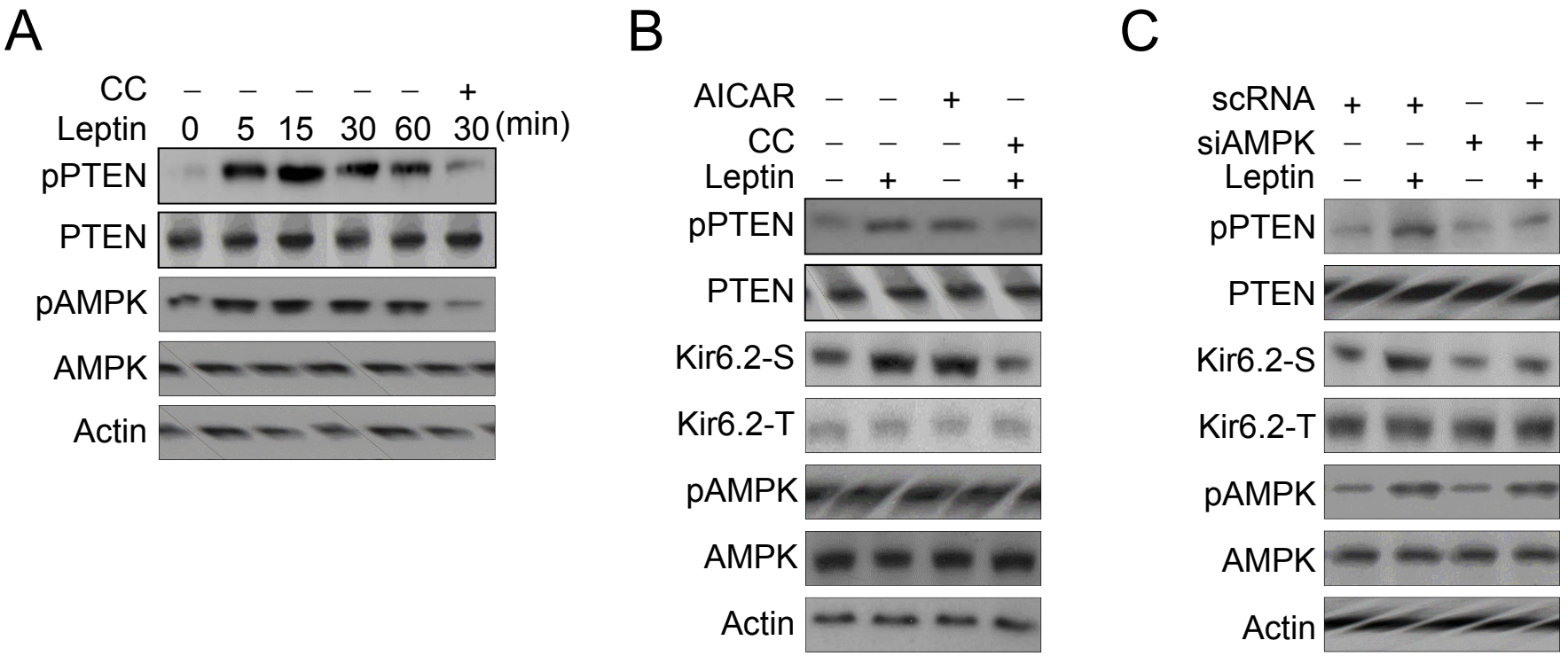


1. Energy status (glucose) and hormonal signal (leptin) are integrated into AMPK activity.
2. AMPK signaling is crucial for normal "metabolism-excitation coupling" in β-cells.

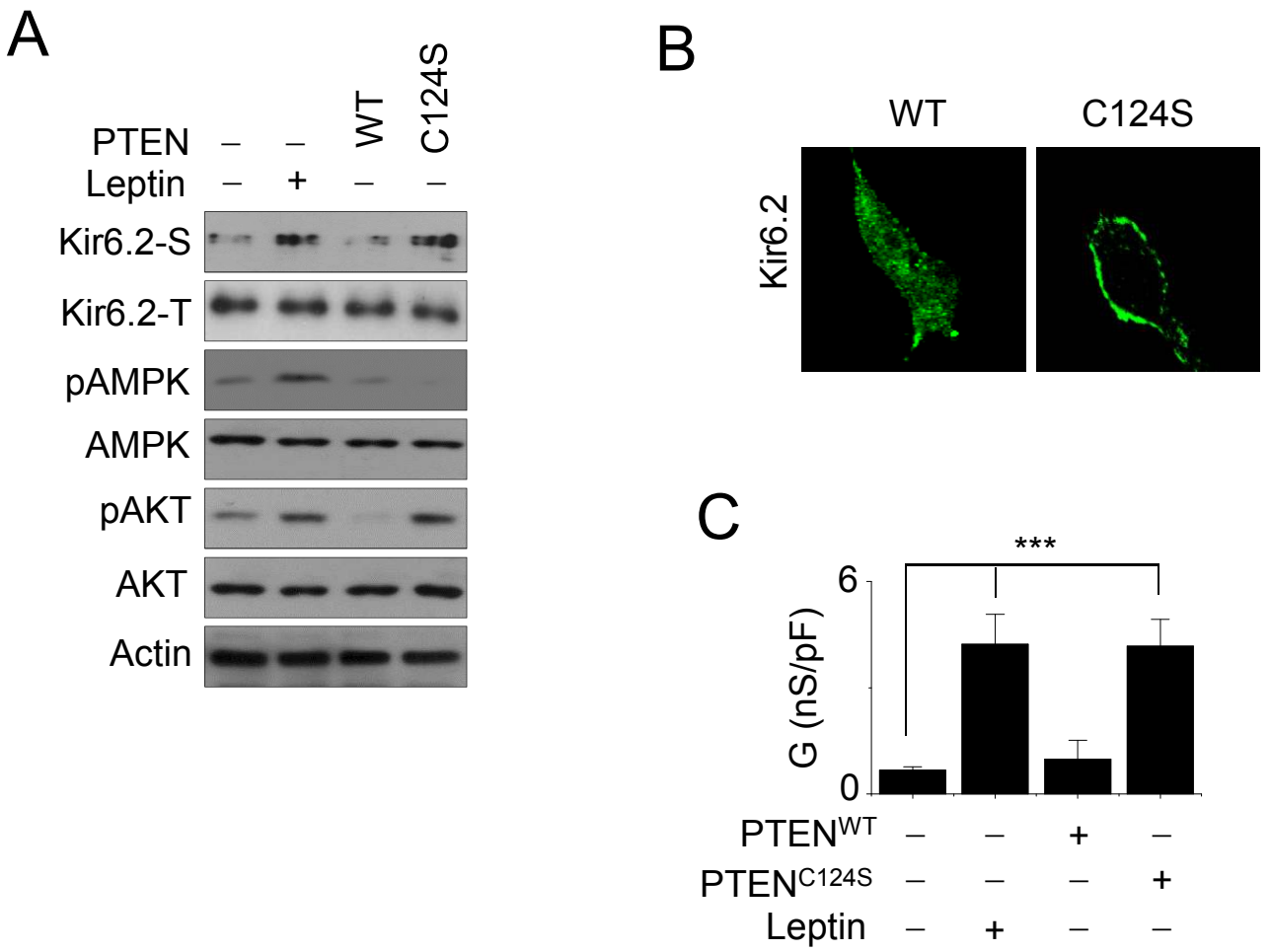
Question 5

- Molecular mechanisms involved in AMPK-dependent channel trafficking?

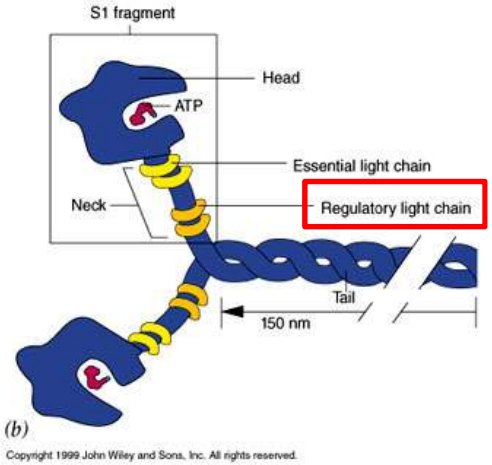
Leptin-induced PTEN inactivation is mediated by AMPK



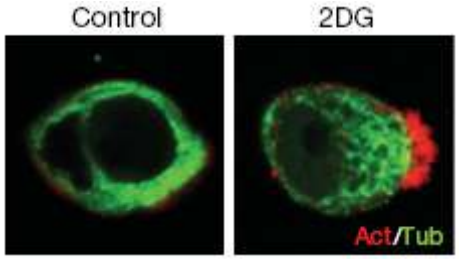
PTEN inactivation is sufficient to promote K_{ATP} trafficking

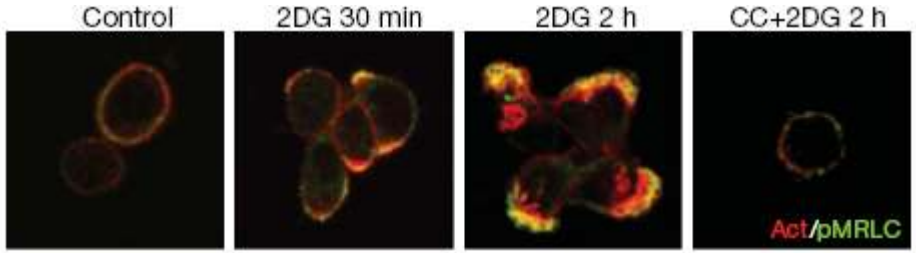


Myosin II and **Myosin regulatory light chain (MRLC)**



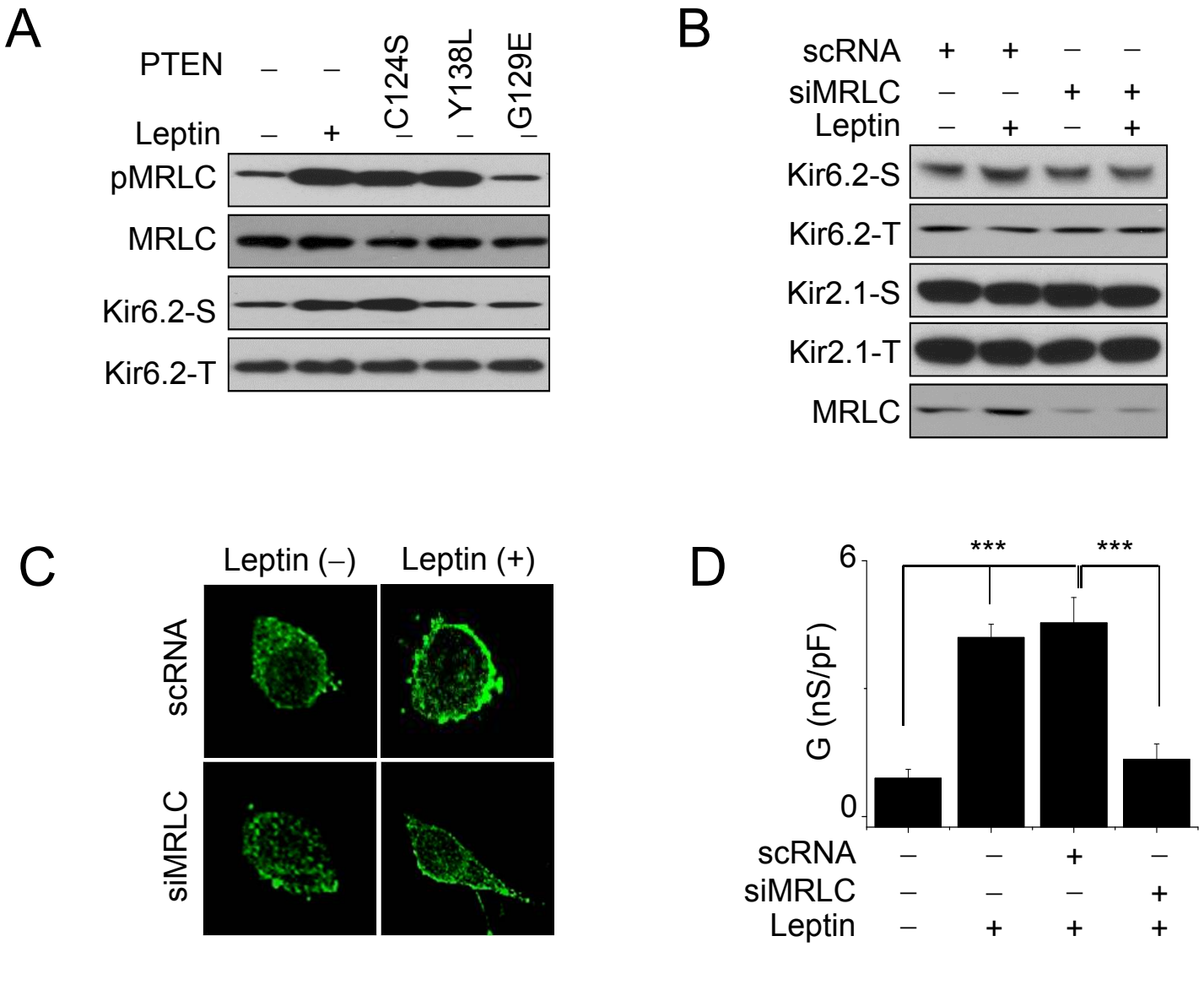
• Myosin II motor activity is regulated by phosphorylation of myosin regulatory light chain (MRLC).



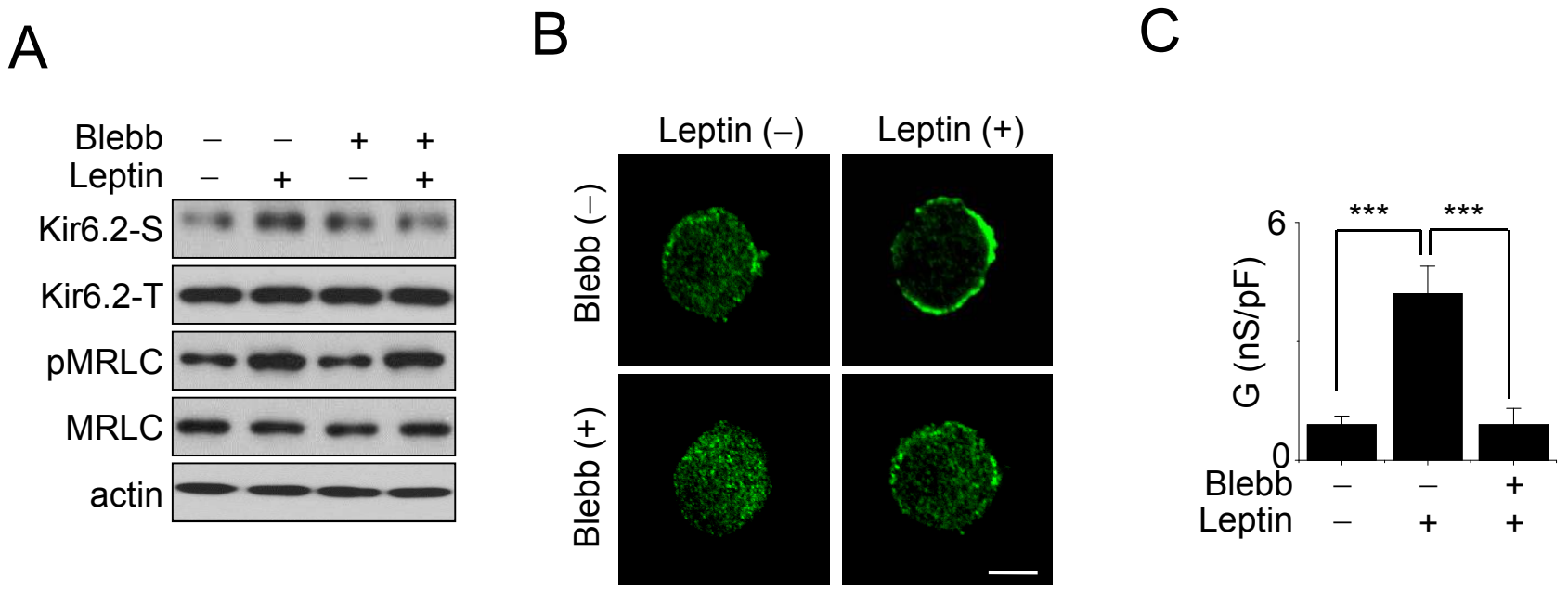


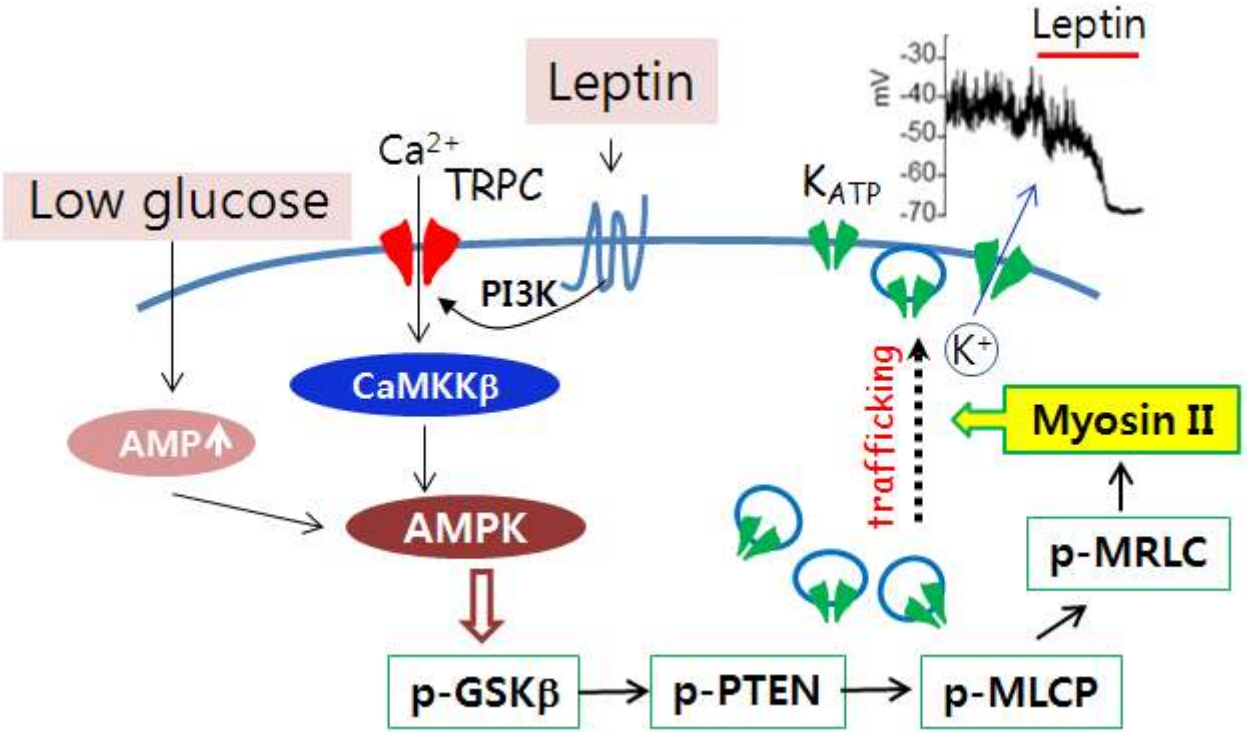
AMPK regulates cell morphology by phosphorylating MRLC in epithelial cells (Lee *et al*., 2007).

Increased MRLC phosphorylation by PTEN inactivation mediates K_{ATP} channel trafficking



Myosin II mediates K_{ATP} channel trafficking




Leptin
Leptin
-30
-40
-50
-60
-70
mV
Low glucose
Ca2+
TRPC
KATP
PI3K
CaMKKβ
AMP
AMPK
K+
trafficking
Myosin II
p-MRLC
p-GSKβ
p-PTEN
p-MLCP

Acknowledgement

-Members of Cell Physiology Lab
(esp. 임아진, 박순현, 지영선, 이석호)
-Collaborators: 전주홍 (서울대), 송대규 (계명대)
Berggren PO (Sweden)