# Cellular Metabolism Pathways

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## *Our Commitment to You*

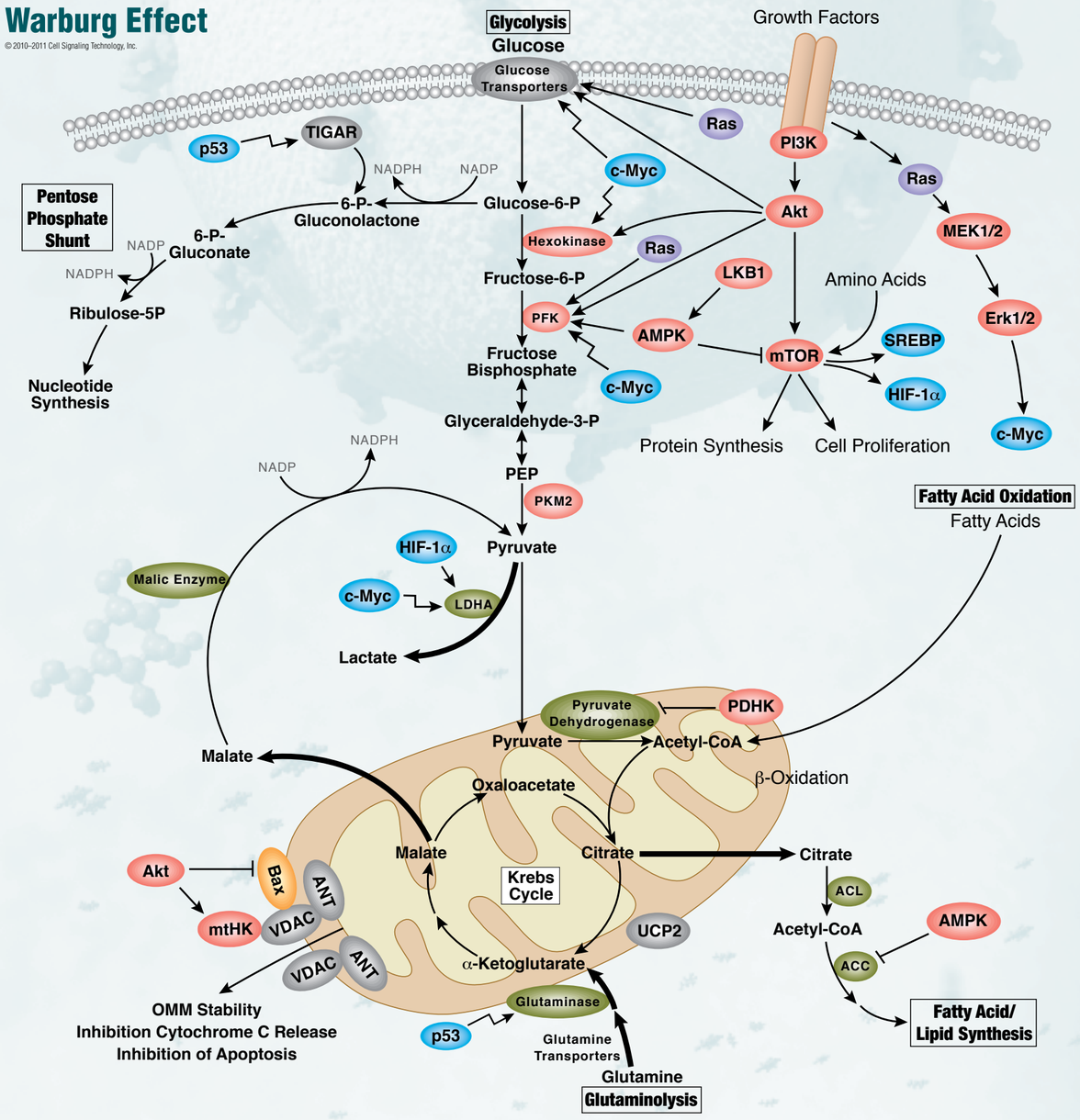
As a company driven by science, our goal is to accelerate biomedical research by developing a "research tool box" that enables researchers to monitor and measure protein activity. We strive to meet contemporary and future research challenges by creating the highest quality, most specific and thoroughly validated antibodies and related reagents.

As a committed member of the research community, we practice responsible and sustainable business methods and invest heavily in research and development. We also encourage thoughtful use of our limited natural resources by highlighting environmental issues in our catalog and by promoting conservation and recycling.

All pathways were created by research scientists at Cell Signaling Technology and reviewed by leading scientists in the field. Visit www.cellsignal.com for additional reference materials and comprehensive validation data for over 3,000 antibodies and related reagents.

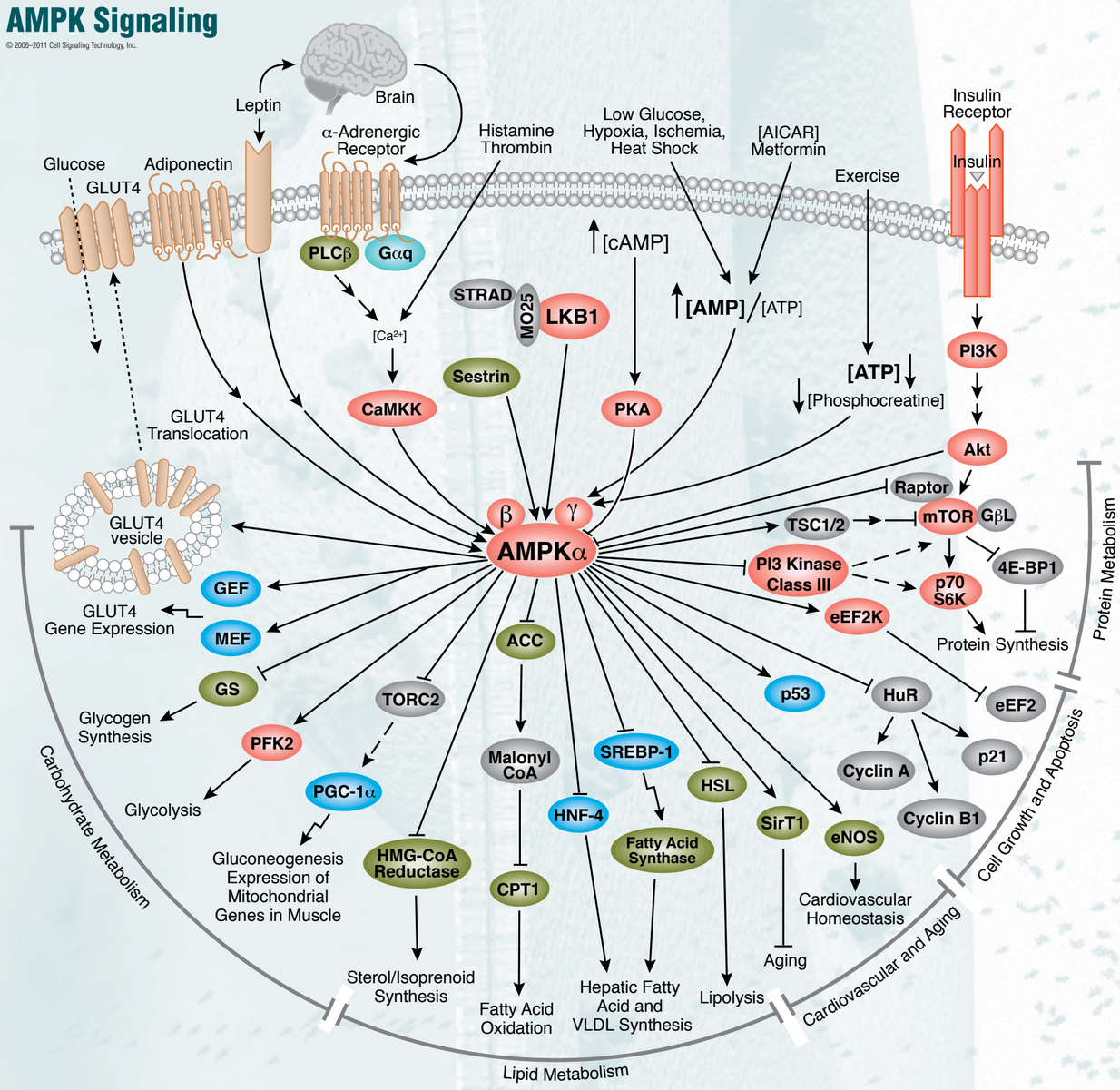
## Warburg Effect





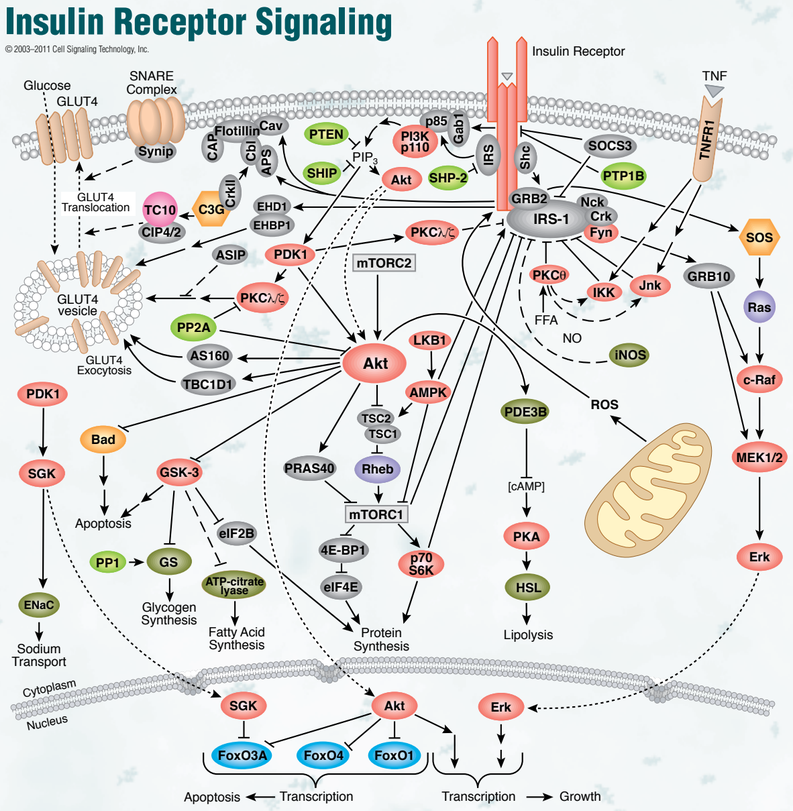

## AMPK Signaling





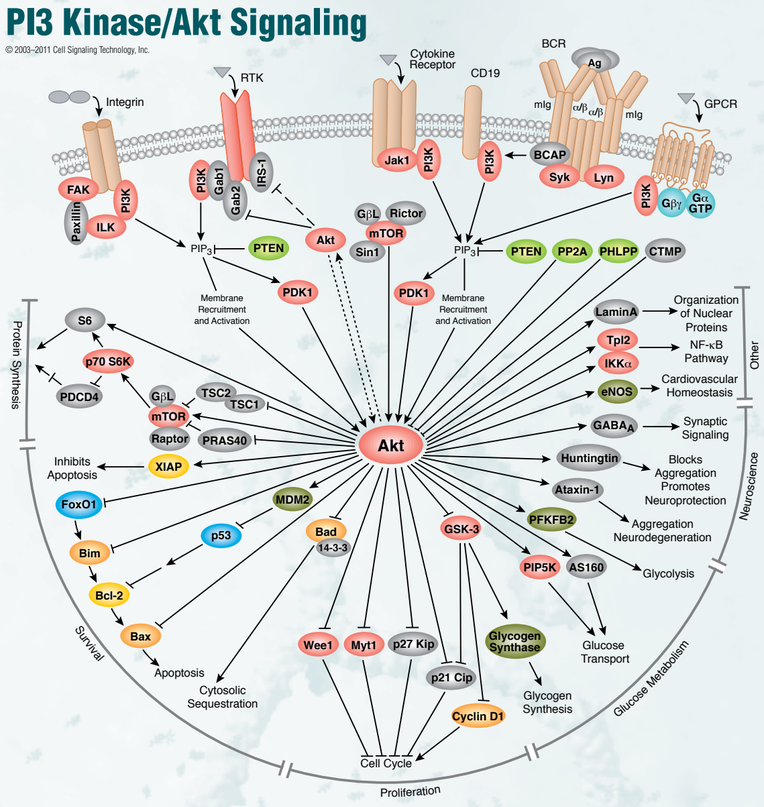

## Insulin Receptor Signaling





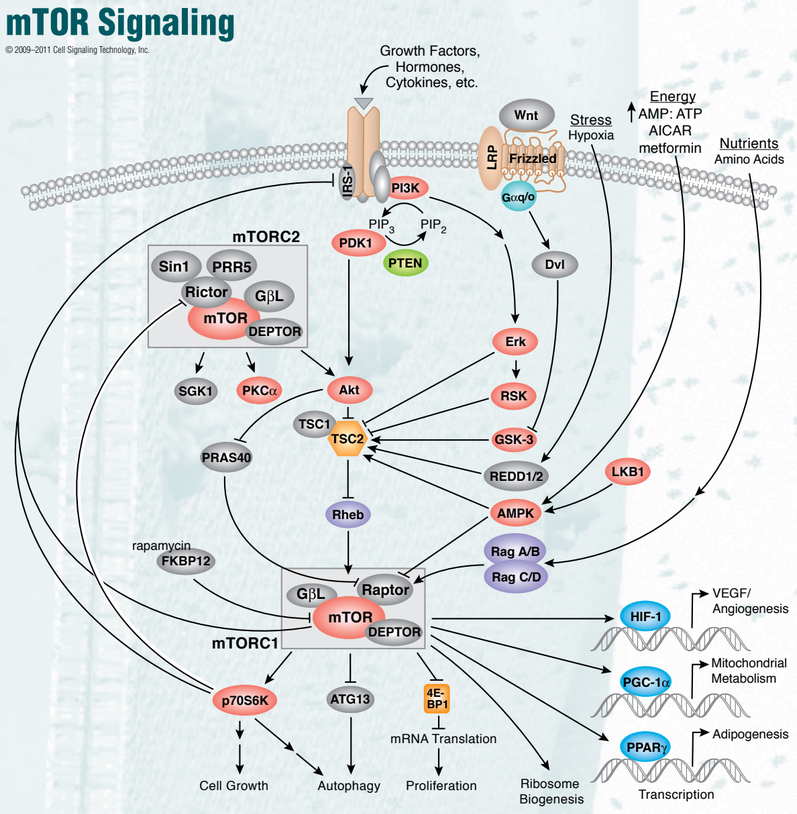

## PI3 Kinase/Akt Signaling






## mTOR Signaling






→ Direct Stimulatory Modification
⊣ Direct Inhibitory Modification
→→ Multistep Stimulatory Modification
→⊣ Multistep Inhibitory Modification
— → Tentative Stimulatory Modification
— ⊣ Tentative Inhibitory Modification
Transcriptional Stimulatory Modification
Transcriptional Inhibitory Modification
Separation of Subunits or Cleavage Products
Joining of Subunits
---→ Translocation
Kinase
Phosphatase
Transcription Factor
Caspase
Receptor
Enzyme
Pro-apoptotic
Anti-apoptotic
GAP
GEF
GTPase
G-protein, ribosomal subunit

### Warburg Effect Pathway Description:

Most cells use glucose as a fuel source. Glucose is metabolized by glycolysis in a multi-step set of reactions resulting in the creation of pyruvate. In typical cells, much of this pyruvate enters the mitochondria where it is oxidized by the Krebs Cycle to generate ATP to meet the cell's energy demands. However, in cancer cells or other highly proliferative cell types, much of the pyruvate from glycolysis is directed away from the mitochondria to create lactate through the action of the enzyme lactate dehydrogenase (LDH). Lactate production is typically restricted to anaerobic conditions when oxygen levels are low, however, cancer cells preferentially channel glucose towards lactate production even when oxygen is plentiful, a process termed "aerobic gycolysis" or the Warburg Effect.

Cancer cells frequently use glutamine as a secondary fuel source, which enters the mitochondria and can be used to replenish Krebs Cycle intermediates or can be used to produce more pyruvate through the action of malic enzyme. Highly proliferative cells need to produce excess lipid, nucleotide, and amino acids for the creation of new biomass. Excess glucose is diverted through the pentose phosphate shunt (PPS) to create nucleotides. Fatty acids are critical for new membrane production and are synthesized from citrate in the cytosol through the action of ATP-citrate lyase (ACL) to generate acetyl-CoA. This process requires NADPH reducing equivalents, which can be generated through the actions of malic enzyme and also from multiple steps within the PPS pathway.

Several signaling pathways contribute to the Warburg Effect. Growth factor stimulation results in signaling through RTKs to activate PI3K/Akt and Ras. Akt promotes glucose transporter activity and stimulates glycolysis through activation of several glycolytic enzymes including hexokinase and phosphofructokinase (PFK). Akt phosphorylation of apoptotic proteins such as Bax makes cancer cells resistant to apoptosis and helps stabilize the outer mitochondrial membrane (OMM) by promoting attachment of mitochondrial hexokinase (mtHK) to the VDAC channel complex. RTK signaling to c-Myc results in transcriptional activation of numerous genes involved in glycolysis and lactate production. The p53 oncogene transactivates TP-53-induced Glycolysis and Apoptosis Regulator (TIGAR) and results in increased NADPH production by PPS.

### AMPK Signaling Pathway Description:

AMP-activated protein kinase (AMPK) plays a key role as a master regulator of cellular energy homeostasis. The kinase is activated in response to stresses that deplete cellular ATP supplies such as low glucose, hypoxia, ischemia, and heat shock. It exists as a heterotrimeric complex composed of a catalytic α subunit and regulatory β and γ subunits. Binding of AMP to the γ subunit allosterically activates the complex, making it a more attractive substrate for its major upstream AMPK kinase, LKB1. Several studies indicate that signaling through adiponectin, leptin and CaMKKβ may also be important in activating AMPK.

As a cellular energy sensor responding to low ATP levels, AMPK activation positively regulates signaling pathways that replenish cellular ATP supplies. For example, activation of AMPK enhances both the transcription and translocation of GLUT4, resulting in an increase in insulin-stimulated glucose uptake. In addition, it also stimulates catabolic processes such as fatty acid oxidation and glycolysis via inhibition of ACC and activation of PFK2. AMPK negatively regulates several proteins central to ATP consuming processes such as TORC2, glycogen synthase, SREBP-1 and TSC2, resulting in the downregulation or inhibition of gluconeogenesis, glycogen, lipid and protein synthesis. Due to its role as a central regulator of both lipid and glucose metabolism, AMPK is considered to be a key therapeutic target for the treatment of obesity, type II diabetes mellitus, and cancer. AMPK is now also recognized as a critical modulator of aging through its interactions with mTOR, SirT1 and the sestrins.

### Insulin Receptor Signaling Pathway Description:

Insulin is the major hormone controlling critical energy functions such as glucose and lipid metabolism. Insulin activates the insulin receptor tyrosine kinase (IR), which phosphorylates and recruits different substrate adaptors such as the IRS family of proteins. Tyrosine phosphorylated IRS then displays binding sites for numerous signaling partners. Among them, PI3K has a major role in insulin function, mainly via the activation of the Akt/PKB and the PKCζ cascades. Activated Akt induces glycogen synthesis, through inhibition of GSK-3; protein synthesis via mTOR and downstream elements; and cell survival, through inhibition of several pro-apoptotic agents (Bad, Forkhead family transcription factors, GSK-3). Insulin stimulates glucose uptake in muscle and adipocytes via translocation of GLUT4 vesicles to the plasma membrane. GLUT4 translocation involves the PI3K/Akt pathway and IR mediated phosphorylation of CAP, and formation of the CAP:Cbl:CrkII complex. Insulin signaling also has growth and mitogenic effects, which are mostly mediated by the Akt cascade as well as by activation of the Ras/MAPK pathway. A negative feedback signal emanating from Akt/PKB, PKCζ, p70 S6K and the MAPK cascades results in serine phosphorylation and inactivation of IRS signaling.

### PI3 Kinases/Akt Signaling Pathway Description:

Since its initial discovery as a proto-oncogene, the serine/threonine kinase Akt (also known as protein kinase B or PKB) has become a major focus of attention because of its critical regulatory role in diverse cellular processes, including cancer progression and insulin metabolism. The Akt cascade is activated by receptor tyrosine kinases, integrins, B and T cell receptors, cytokine receptors, G protein coupled receptors and other stimuli that induce the production of phosphatidylinositol 3,4,5 triphosphates (PtdIns(3,4,5)P3) by phosphoinositide 3-kinase (PI3K). These lipids serve as plasma membrane docking sites for proteins that harbor pleckstrin-homology (PH) domains, including Akt and its upstream activator PDK1. There are three highly related isoforms of Akt (Akt1, Akt2, and Akt3) and these represent the major signaling arm of PI3K. For example, Akt is important for insulin signaling and glucose metabolism, with genetic studies in mice revealing a central role for Akt2 in these processes. Akt regulates cell growth through its effects on the mTOR and p70 S6 kinase pathways, as well as cell cycle and cell proliferation through its direct action on the CDK inhibitors p21 and p27, and its indirect effect on the levels of cyclin D1 and p53. Akt is a major mediator of cell survival through direct inhibition of pro-apoptotic signals such as Bad and the Forkhead family of transcription factors. T lymphocyte trafficking to lymphoid tissues is controlled by the expression of adhesion factors downstream of Akt. In addition, Akt has been shown to regulate proteins involved in neuronal function including GABA receptor, ataxin-1, and huntingtin proteins. Akt has been demonstrated to interact with Smad molecules to regulate TGFβ signaling. Finally, lamin A phosphorylation by Akt could play a role in the structural organization of nuclear proteins. These findings make Akt/PKB an important therapeutic target for the treatment of cancer, diabetes, laminopathies, stroke and neurodegenerative disease.

### mTOR Signaling Pathway Description:

The mammalian target of rapamycin (mTOR) is an atypical serine/threonine kinase that is present in two distinct complexes. mTOR complex 1 (mTORC1) is composed of mTOR, Raptor, GβL (mLST8), and Deptor and is partially inhibited by rapamycin. mTORC1 integrates multiple signals reflecting the availability of growth factors, nutrients, or energy to promote either cellular growth when conditions are favorable or catabolic processes during stress or when conditions are unfavorable. Growth factors and hormones (e.g. insulin) signal to mTORC1 via Akt, which inactivates TSC2 to prevent inhibition of mTORC1. Alternatively, low ATP levels lead to the AMPK-dependent activation of TSC2 to reduce mTORC1 signaling. Amino acid availability is signaled to mTORC1 via a pathway involving the Rag proteins. Active mTORC1 has a number of downstream biological effects including translation of mRNA via the phosphorylation of downstream targets (4E-BP1 and p70 S6 Kinase), suppression of autophagy, ribosome biogenesis, and activation of transcription leading to mitochondrial metabolism or adipogenesis. The mTOR complex 2 (mTORC2) is composed of mTOR, Rictor, GβL, Sin1, PRR5/Protor-1, and Deptor and promotes cellular survival by activating Akt. mTORC2 also regulates cytoskeletal dynamics by activating PKCα and regulates ion transport and growth via SGK1 phosphorylation. Aberrant mTOR signaling is involved in many disease states including cancer, cardiovascular disease, and metabolic disorders.

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