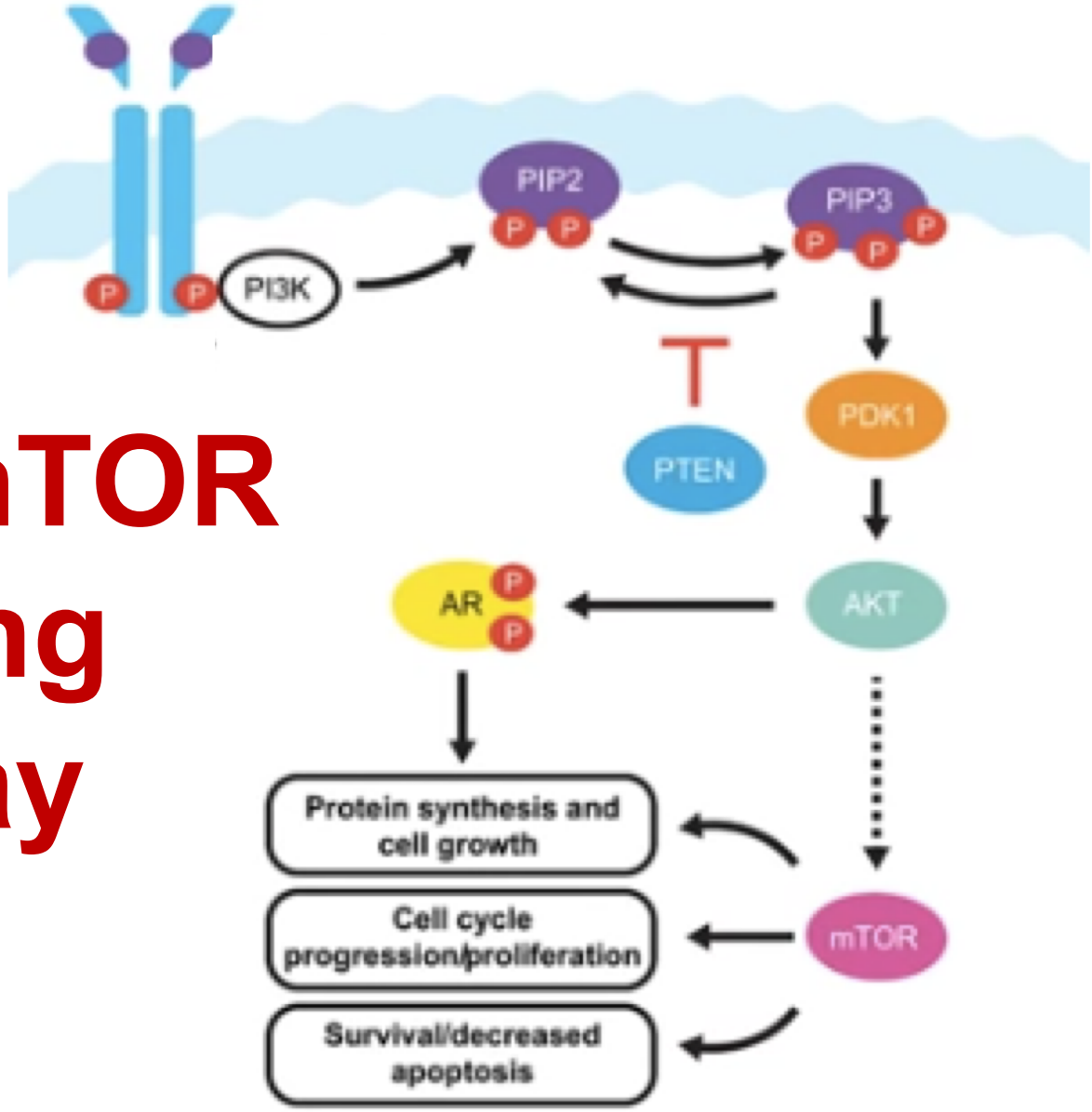
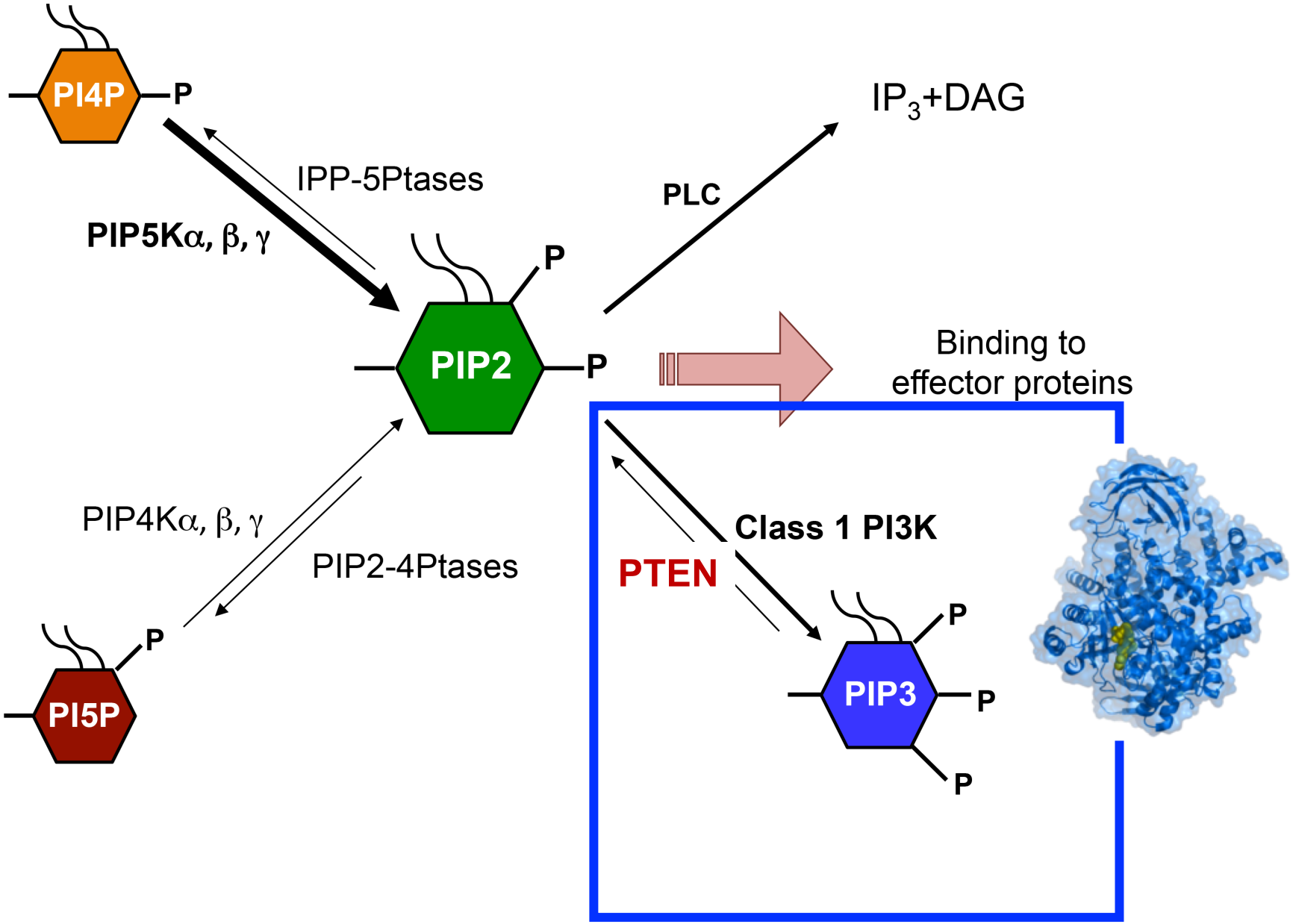
PI3K/Akt/mTOR signalling pathway

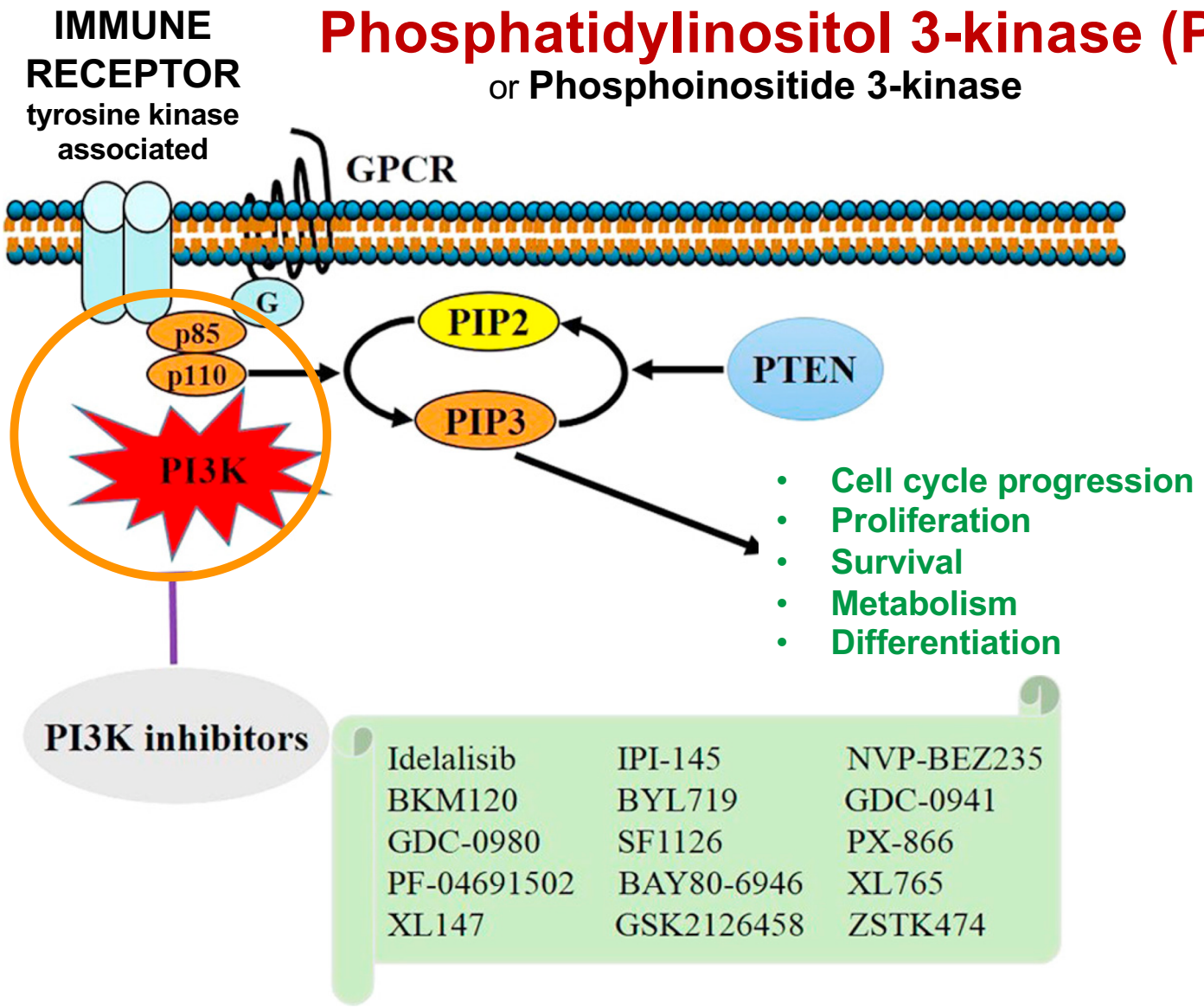


Phosphatidylinositol 4,5-bisphosphate (PIP2)



Phosphatidylinositol 3-kinase (PI3K)

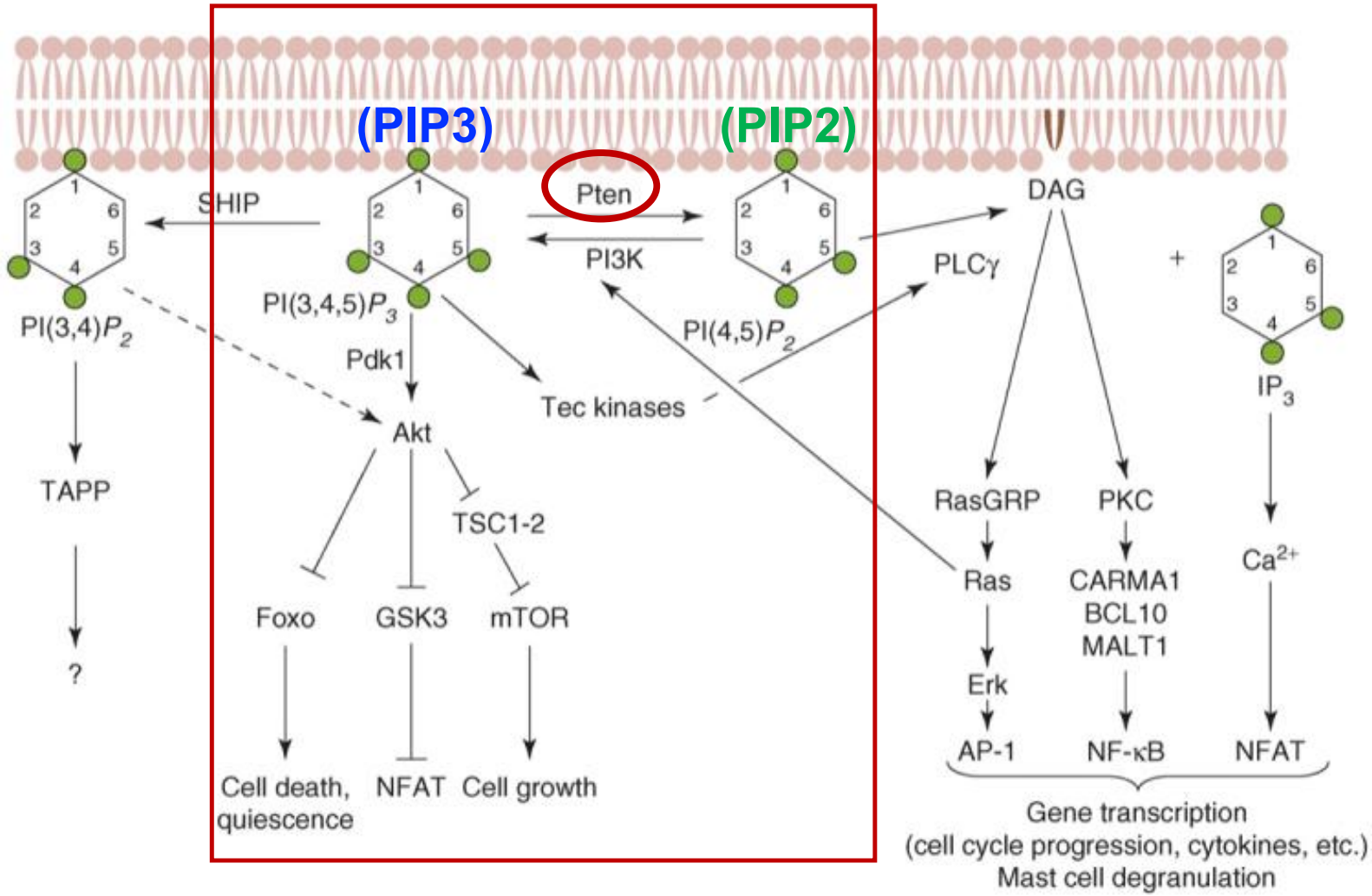
or **Phosphoinositide 3-kinase**



- Discovered in 1985
- Is one of the major effectors downstream of tyrosine kinase-associated immune receptors and G protein-coupled receptors (chemokine receptors)→regulates cell survival, proliferation, metabolism and differentiation.
- In the immune system, impaired PI3K signalling leads to **immunodeficiency**, while aberrant PI3K signalling contributes to **autoimmunity** and **leukaemia**.

- Generates **phospholipids, activates Akt** (**PKB**) and **mTOR.**
- The most commonly activated signalling pathway in several cells of the immune system.

PTEN: Tumor Suppressor and Metabolic Regulator

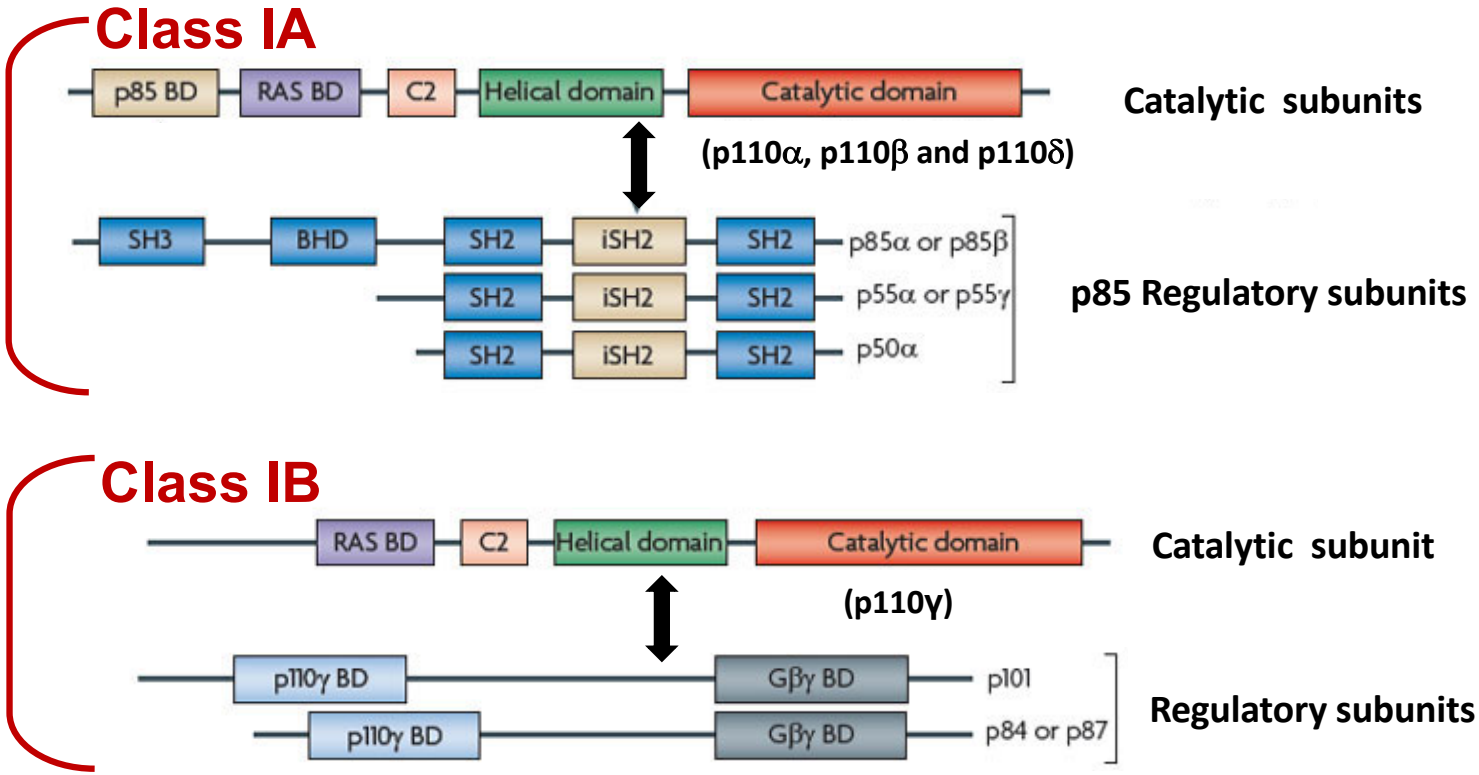


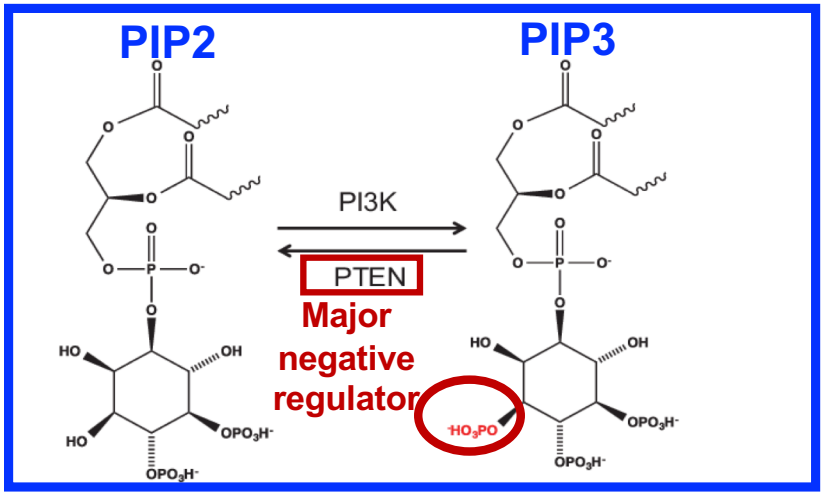
The cellular levels of PtdIns(3,4,5)P3 **(PIP3)** are tightly regulated by the opposing activity of **PTEN** (Phosphatase and tensin homolog) a **lipid phosphatase** that antagonizes PI3K activity by converting PIP3 back to phosphatidylinositol-4,5-bisphosphate **(PIP2).**

PTEN (phosphatase and tensin homologue)

PI3K family is divided into different classes:
Class I (A and B); Class II; Class III; Class IV

PIP2 → PIP3
(PtdIns(4,5)P_2 → PtdIns(3,4,5)P_3)



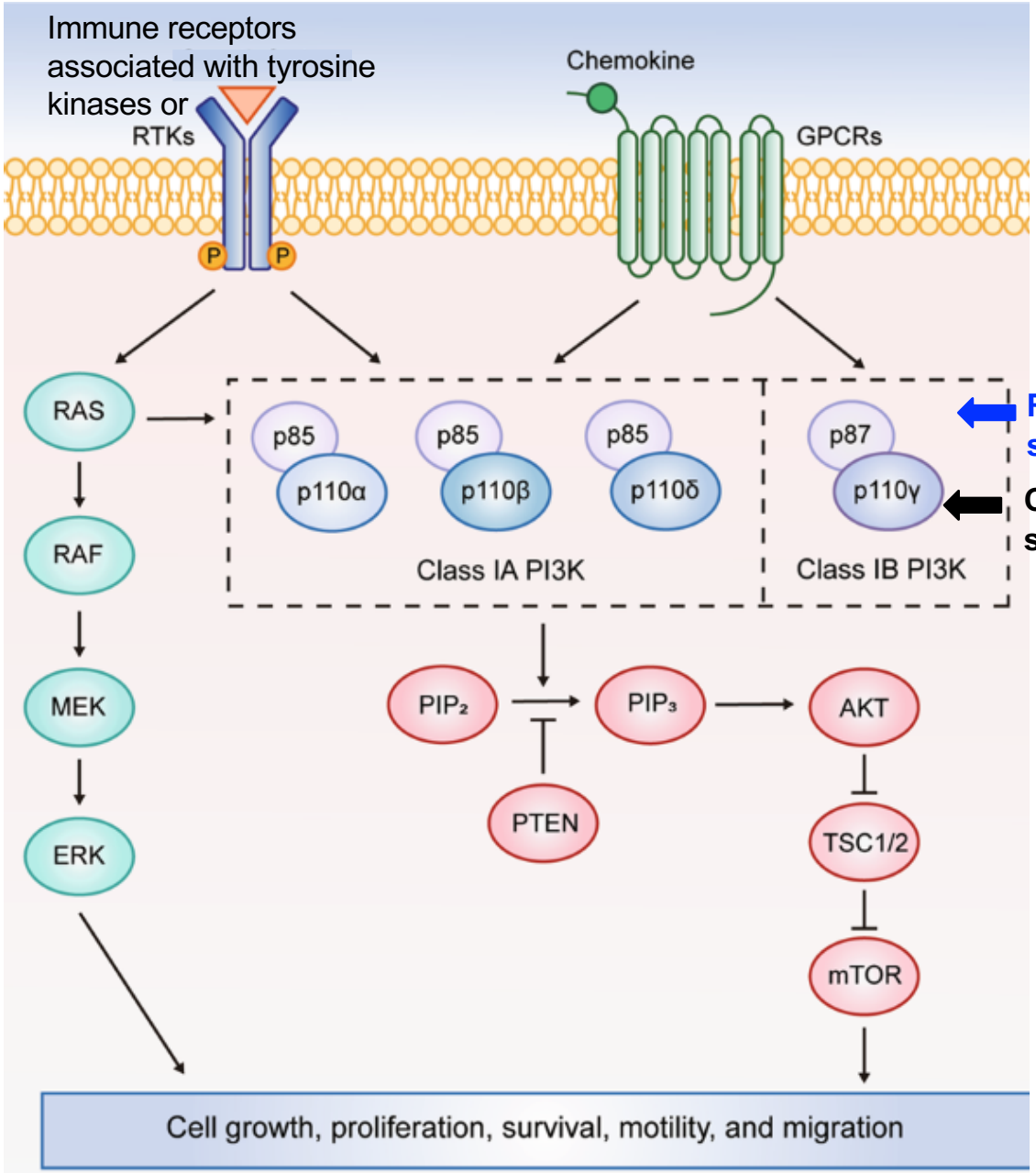


Activation:

Class IA by: -Immune receptors associated with tyrosine kinases
-GPCRs (Chemokine receptors)
-Ras

Class IB by: -GPCRs

Activation of Class IA and IB PI3K



- Class I PI3K isoforms are heterodimers consisting of **p110 (catalytic subunit)** and **p85** or **p87** or **p101 (regulatory subunits).**

- Class IA PI3Ks can be activated by RTKs, GPCRs, RAS and other adapter proteins, while class IB PI3K is exclusively activated by GPCRs.

- When PI3K is activated by upstream signals, PIP_3 is generated from PIP_2 and activates downstream signaling pathways, such as the **AKT/mTOR pathway**. The activated PI3K pathway ultimately contributes to cell growth, proliferation, survival, motility and migration.

PI3K family is divided into different classes: Class I (A and B)

Class II

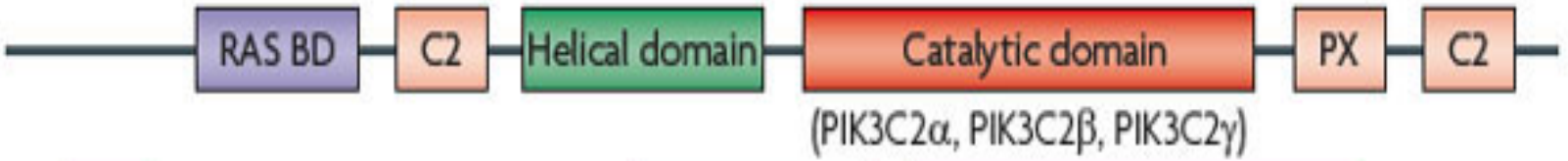
Class III

Class IV

PtdIns → PtdIns(3)P

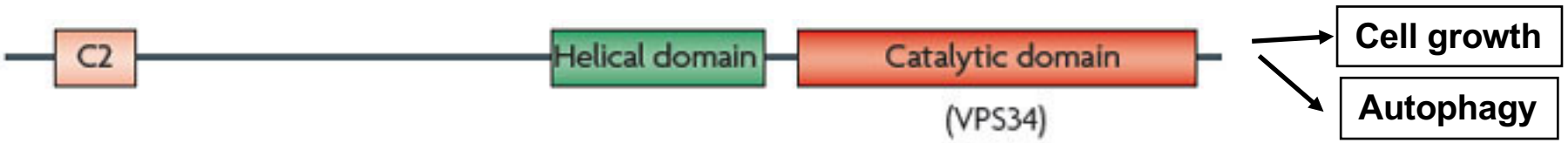
PtdIns(4)P → PtdIns(3,4)P_2

Class II

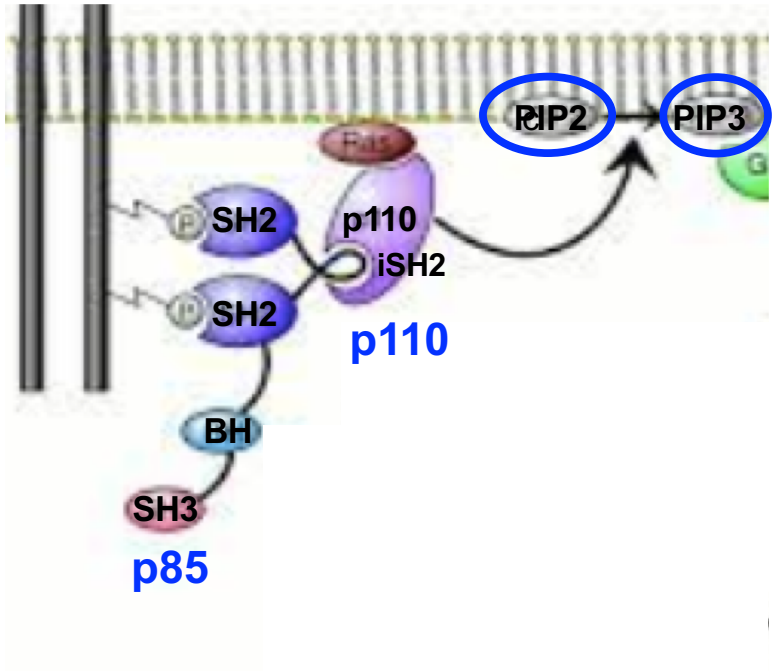


PtdIns → PtdIns(3)P

Class III



Class I PI3K



Catalytic subunits:

PI3KCA → **p110α**,
PI3KCB → **p110β**,
PI3KCD → **p110δ**;
(class I A)

PIK3CG → **p110γ** (class I B)

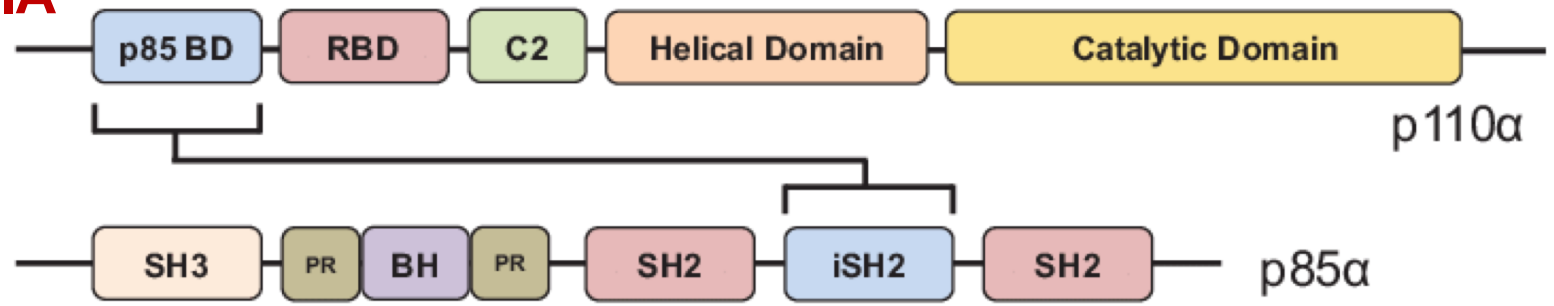
- **p110α, p110β** are **ubiquitously expressed.**
- **p110γ** and **p110δ** are preferentially expressed in cells of hematopoietic origin (immune system).

Regulatory subunits:

PIK3R1 encodes **p85α** (and its splice variants p55α and p50α);
PIK3R2 → **p85β**;
PIK3R3 → **p55γ**; (class I A)

PIK3R5 → **p101**; PIK3R6 → **p87, p84** (class I B)

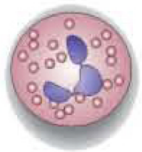
CLASS IA



Structure and biochemistry of PI3K. The domains of PI3K catalytic (p110a) and regulatory (p85a) subunits are represented. The connecting arrow indicates the domains involved in the interaction between these 2 subunits. BD (Binding Domain), RBD (Ras-BD), SH3 (SRC Homology 3), PR (Proline-Rich), BH (BcR Homology), SH2 (SRC Homology 2), iSH2 (inter-SH2).

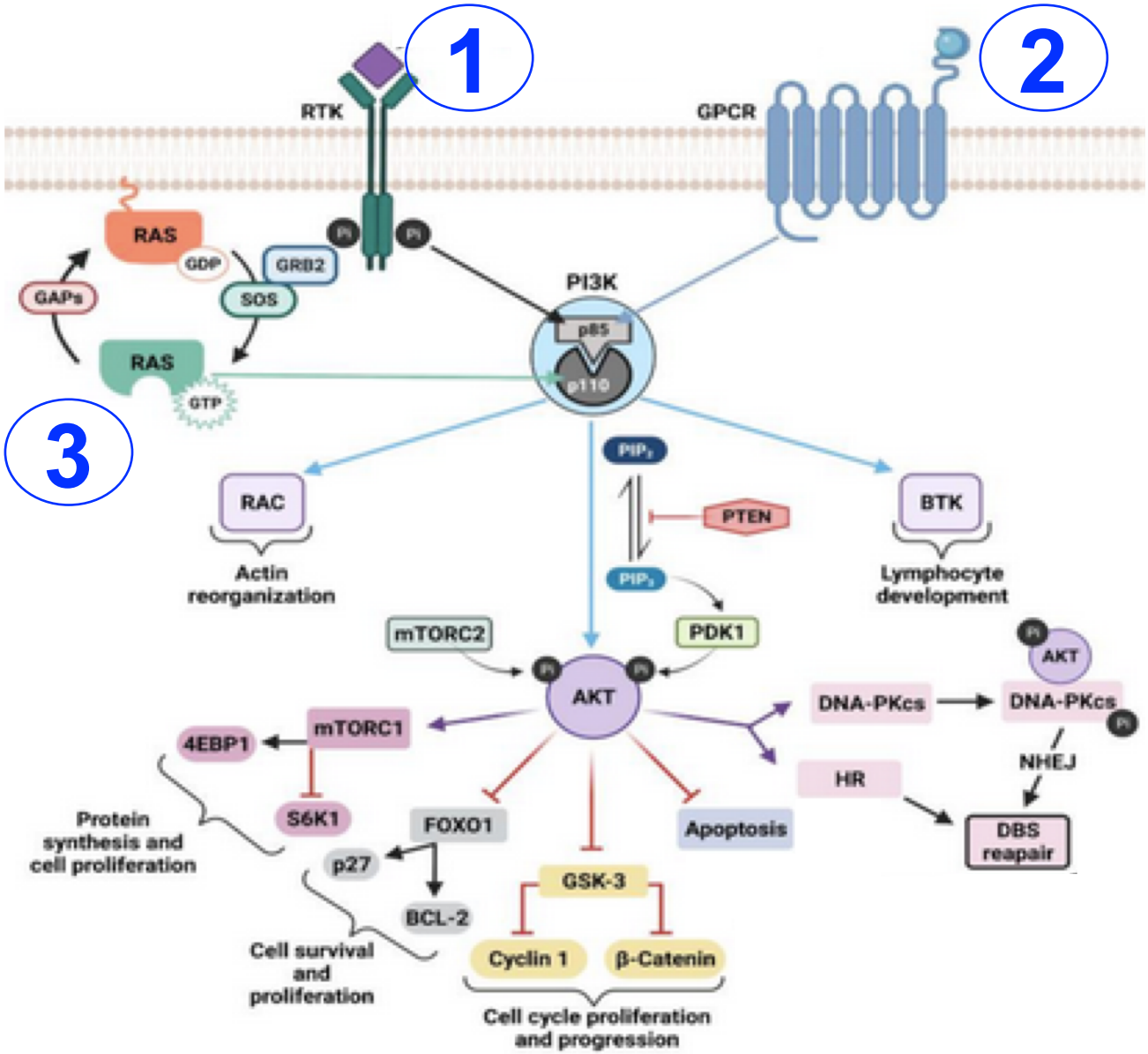
Role of class I PI3K in adaptive and innate immunity

Roles of PI3Kγ and PI3Kδ in innate and adaptive immune system

Cell type	PI3Kγ	PI3Kδ
 Neutrophils	Chemotaxis ROS production	Chemotaxis
 Macrophages		
 Mast cells	Mast cell degranulation (late phases)	Mast cell degranulation (early phases)
 Eosinophils	Eosinophil migration	
 T lymphocytes	Development (thymocyte maturation) Proliferation and cytokine production Immunological synapse organization	Differentiation and expansion of Th1, Th2, Th17, and Treg Lymph node-homing
 B lymphocytes		Development and proliferation Antibody production Immunoglobulin class switch

Ghigo et al BioEssays 2010

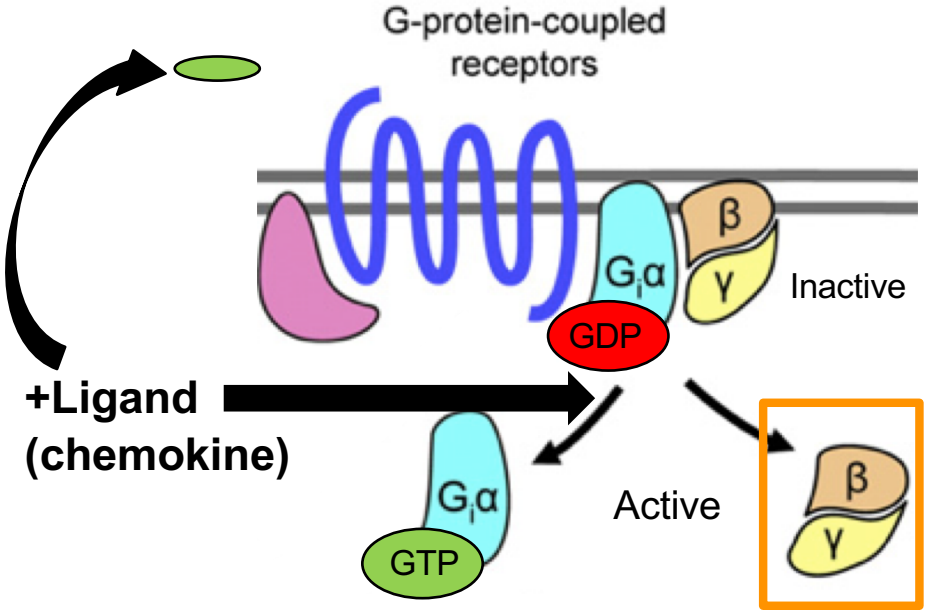
Mechanisms of activation of PI3K and downstream effectors

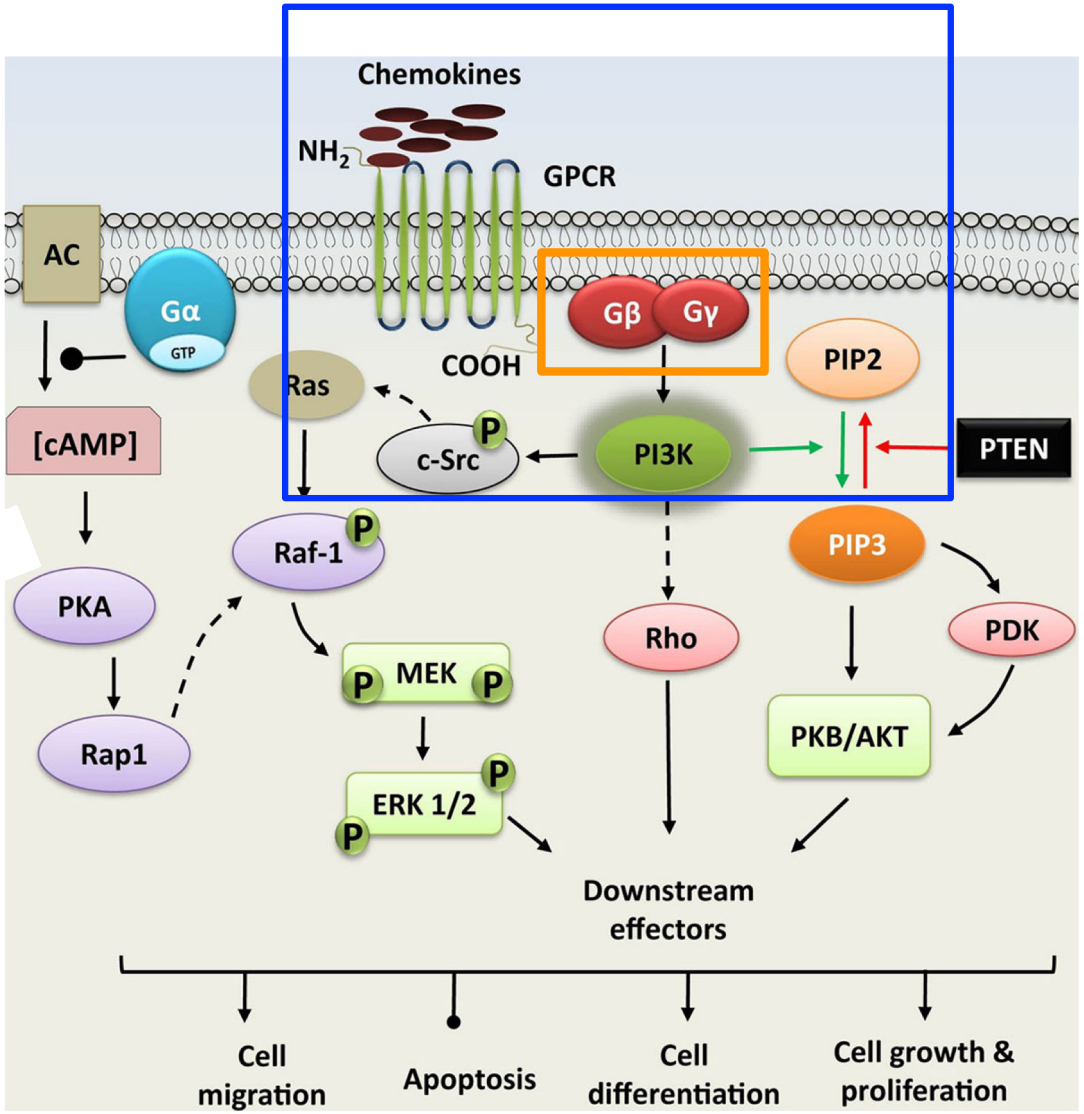


GCPRs and RTKs are upstream signals that control PI3K activation through direct interaction with the regulatory subunit of PI3K.

Further, RTK can activate PI3K indirectly through **Ras activation that in turn activates PI3K in a p110-dependent manner.** Once activated, PI3K generates PIP_3 that promotes AKT phosphorylation, which subsequently phosphorylates a large number of downstream targets to control cell survival, proliferation and apoptosis. Other PI3K effectors are TEC family tyrosine kinase, such as BTK, and GTPases of the Rho/Rac/cdc42 family.

Chemokine receptors



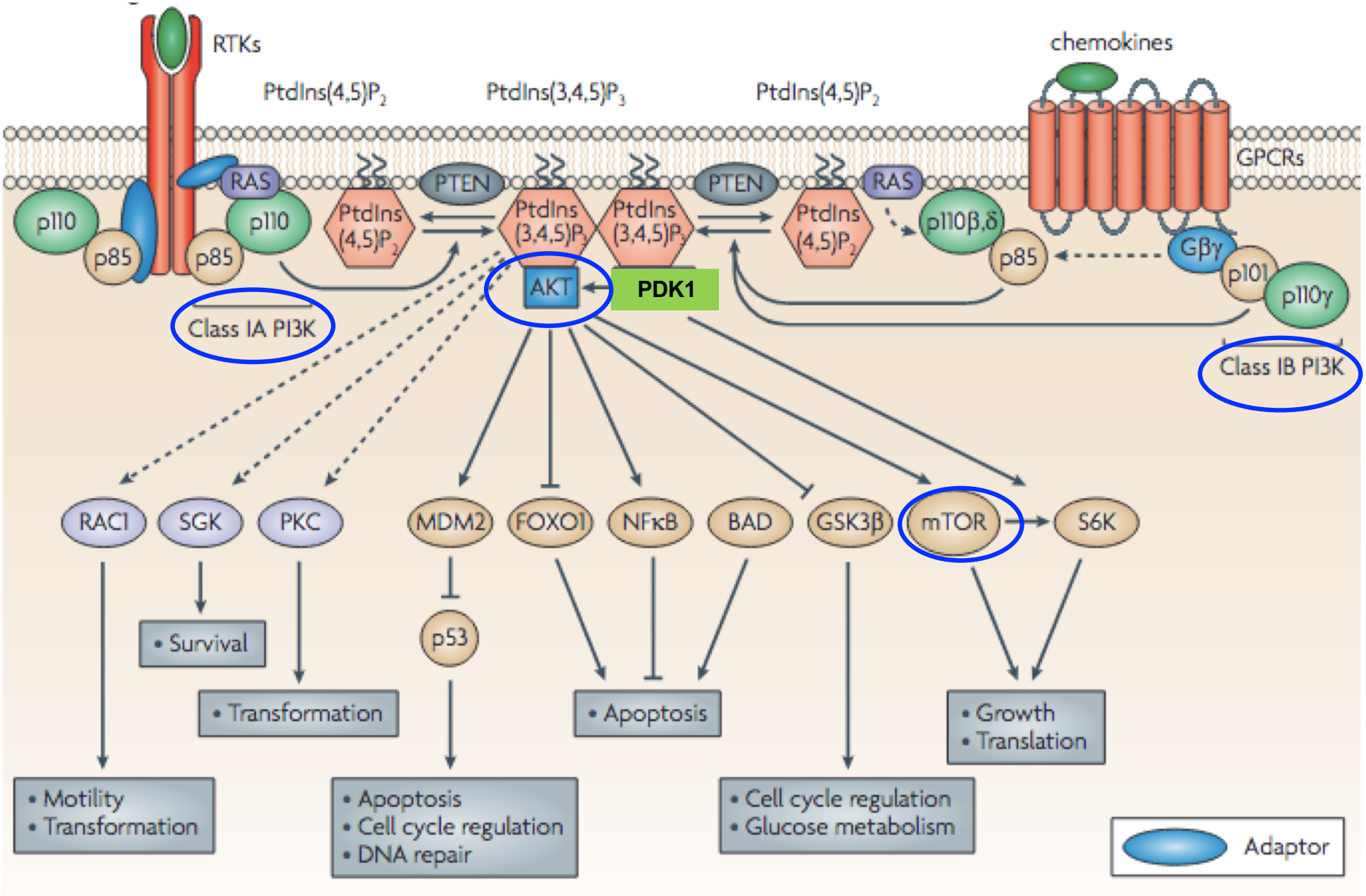


PI3K pathway is activated upon agonist binding to receptor G protein coupled receptors (GPCRs). GPCRs can activate PI3Ks via G proteins, such as **Gβγ**.

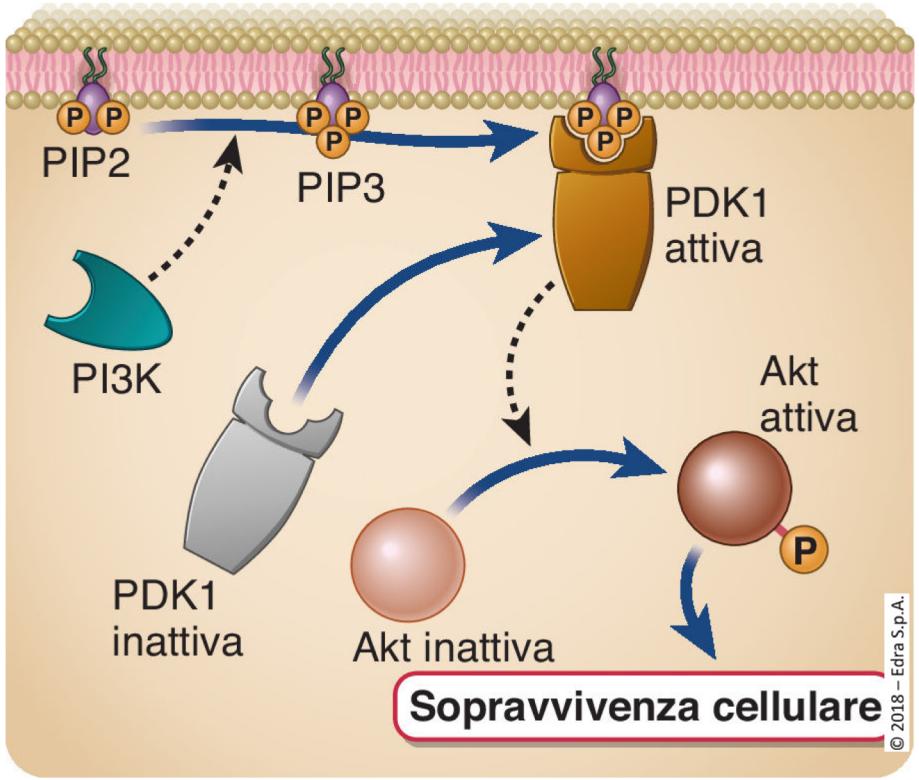
PI3K phosphorylates the phosphatidylinositol (3, 4)-bisphosphate (PIP2), generating phosphatidylinositol (3, 4, 5)-trisphosphate (PIP3) which recruits other kinases like serine/threonine kinase (PDK1/AKT).

This signaling pathway modulates cellular functions, including proliferation, gene expression, cytoskeletal rearrangement, anti-apoptosis, and degranulation.

Class 1 PI3K pathway



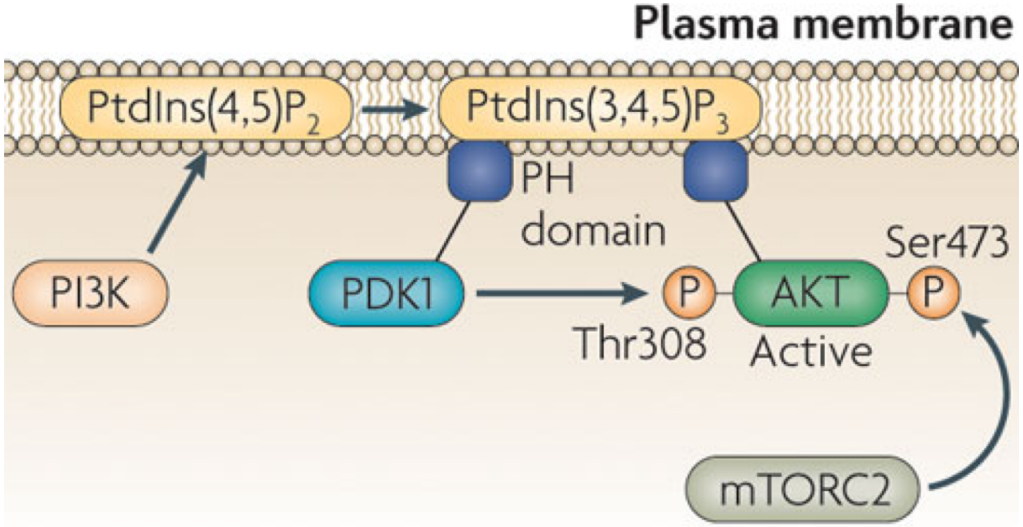
Attivazione di Akt/PKB da parte di PI3K



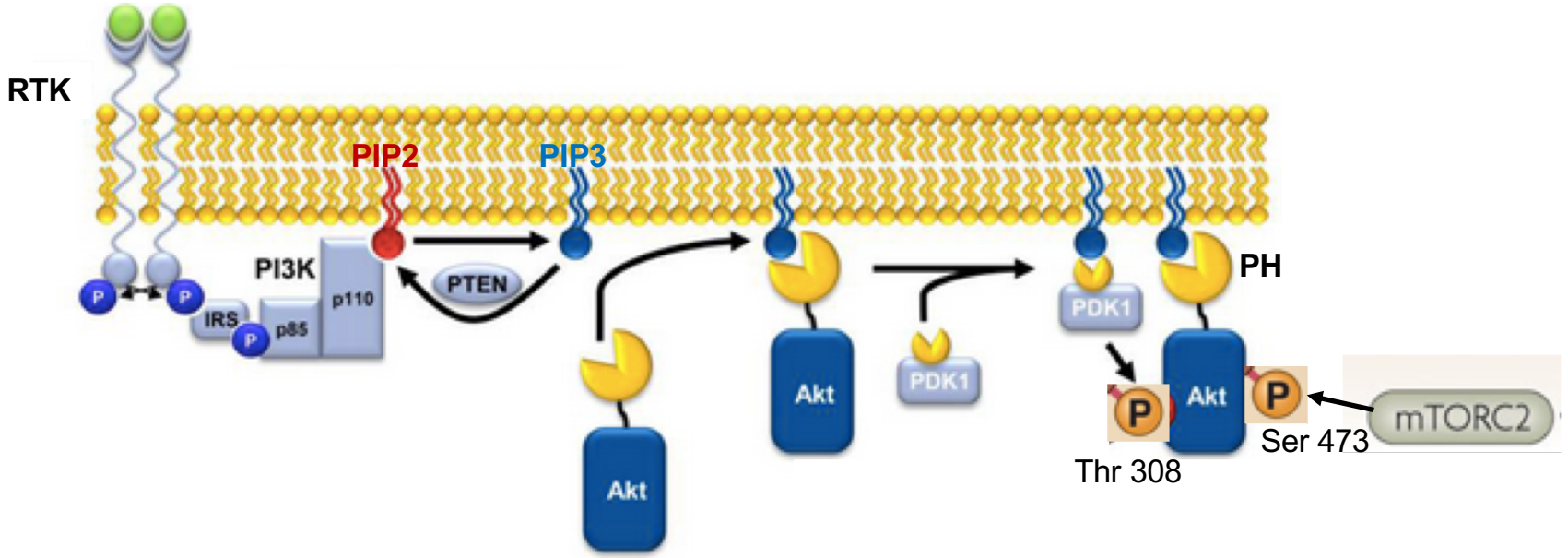
PIP3 recluta PDK1 e Akt permettendo a PDK1 di fosforilare ed attivare Akt

AKT fosforila numerosi geni target (attivandoli o inattivandoli) che hanno molteplici effetti tra cui sopravvivenza cellulare

PDK1 = Phosphoinositide-dependent kinase-1



Attivazione di Akt/(PKB) da parte di PI3K



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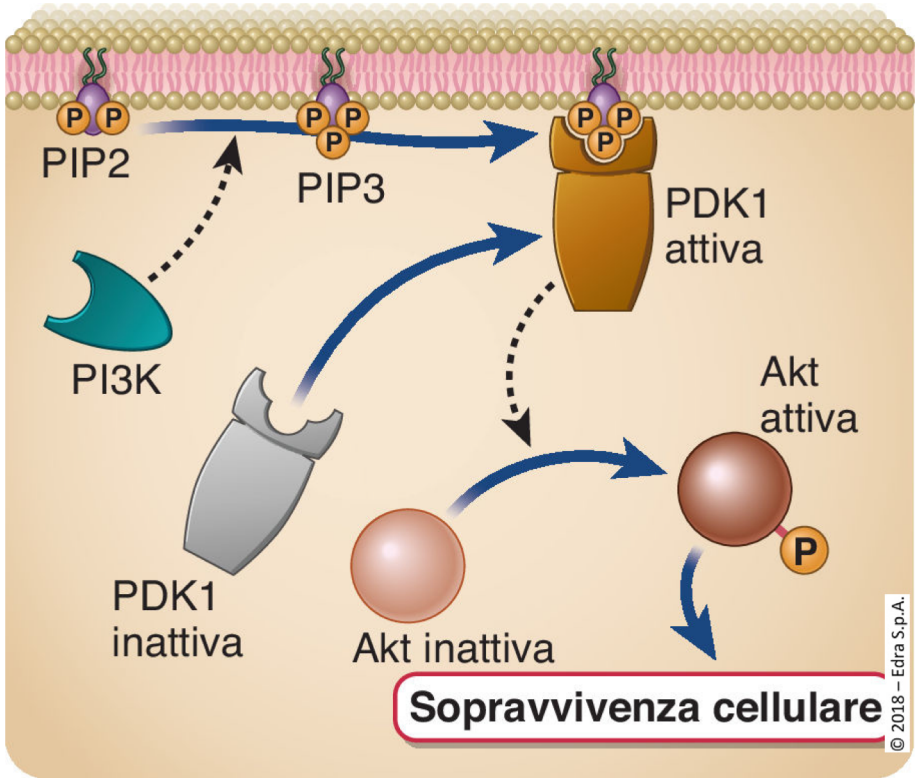
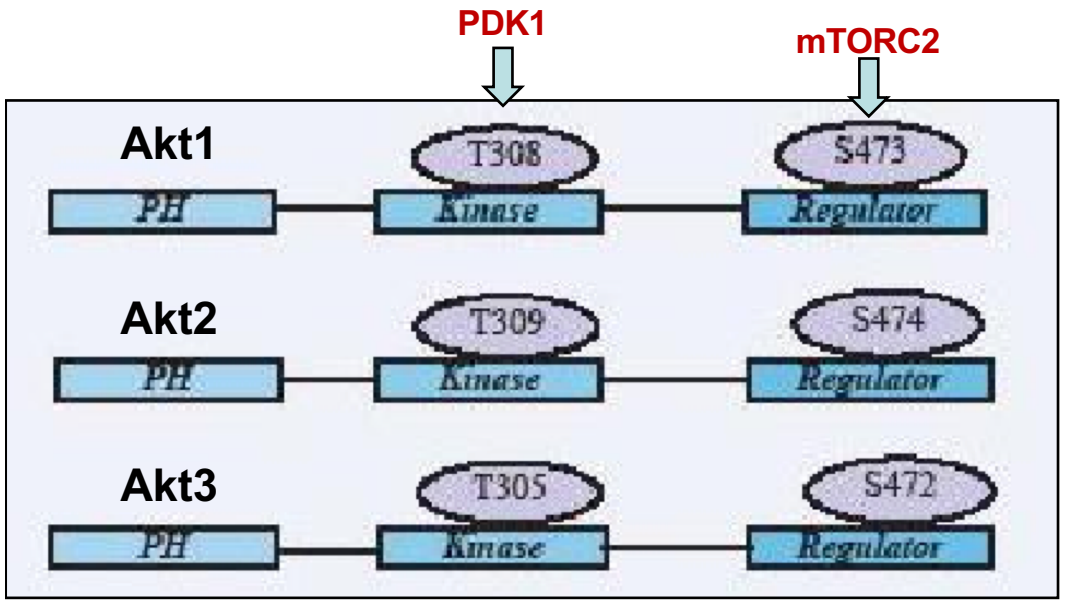


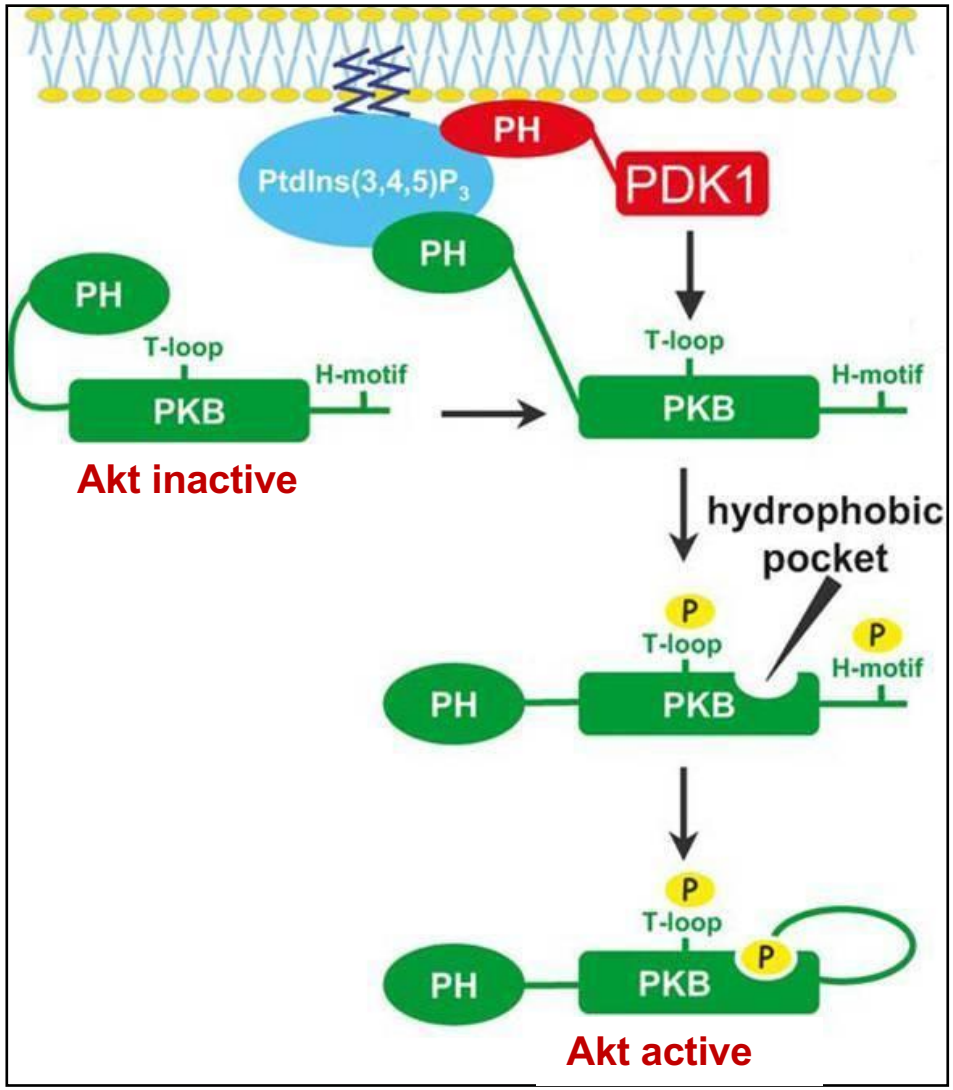
PDK1 = Phosphoinositide-dependent kinase-1

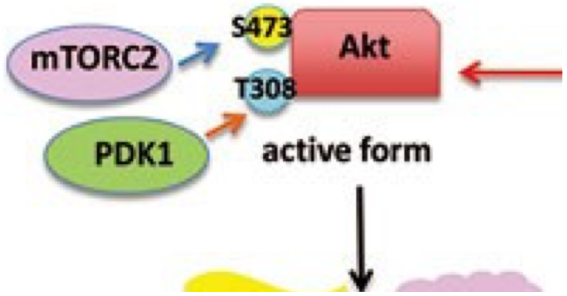
Akt/PKB activation

- Akt/PKB was firstly discovered as an oncogene (**v-akt**) of an acute transforming retrovirus (**AKT8**)
- **Akt1, Akt 2 and Akt3**: Ser/Thr kinases encoded by **PKBα**, **PKBβ**, **PKBγ** genes, respectively



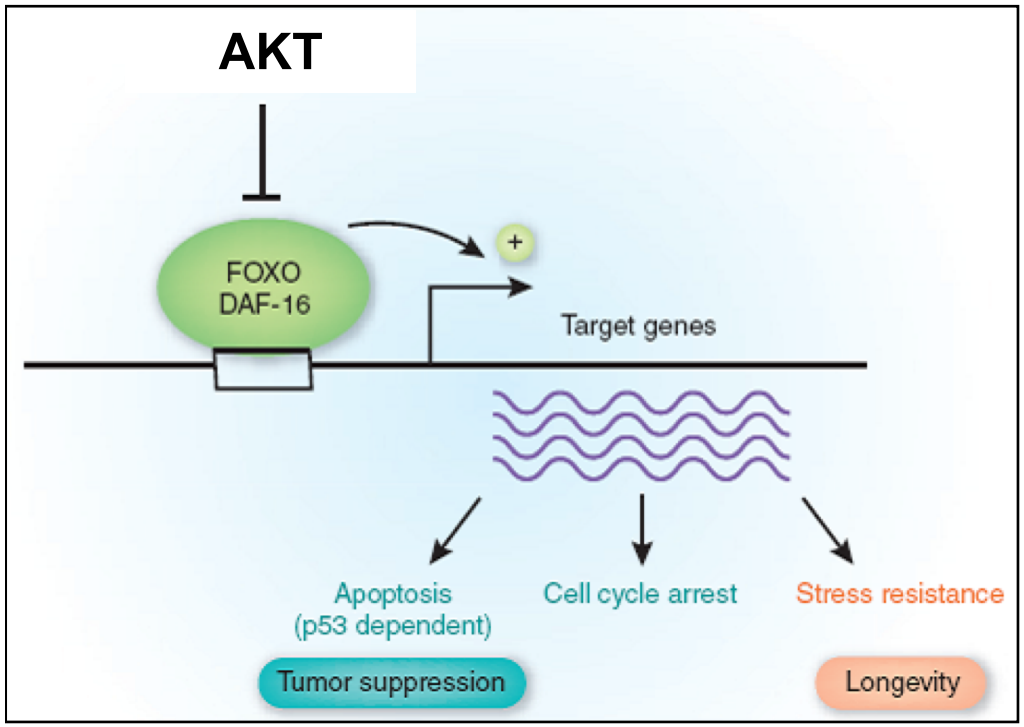
- **mTORC2** phosphorylates **S473**, 474 or 472
- **Phosphoinositide-dependent Kinase 1 (PDK1)** phosphorylates **T308**, **309** or **305**



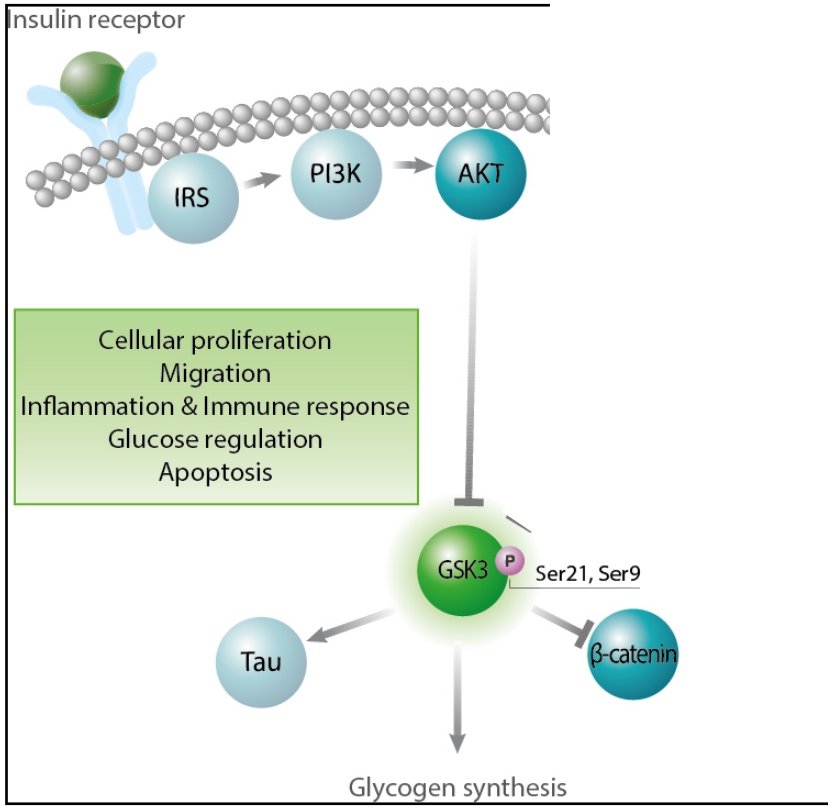


Cell survival

Direct effects of Akt activation



autophagy
ubiquitin-proteasome system



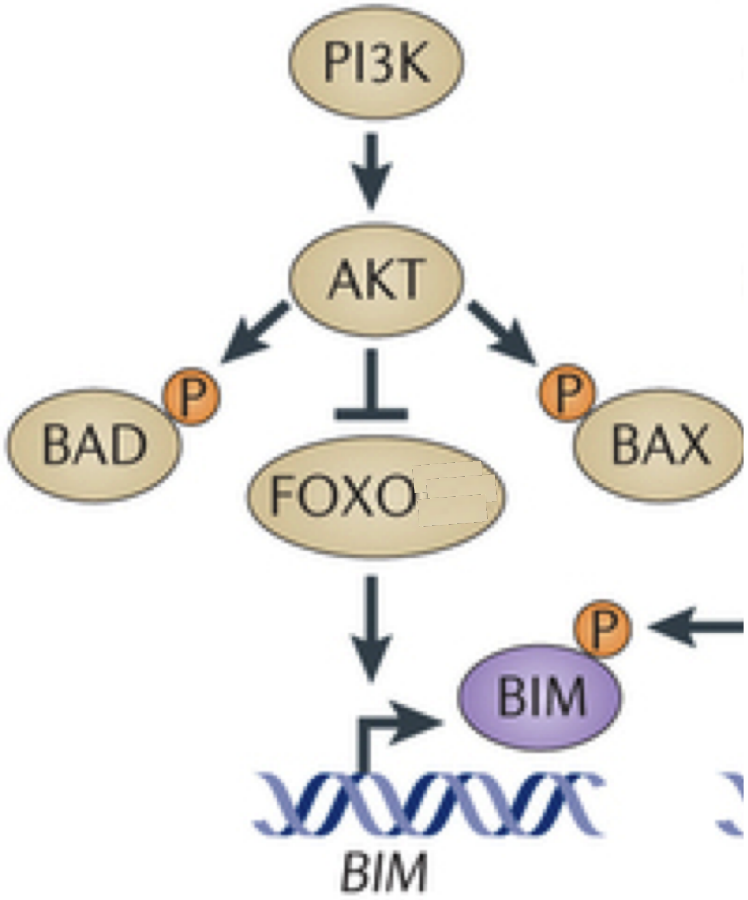
Akt directly phosphorylates FOXO (Forkhead box O) thus inducing the **inhibition** of its transcription functions

FOXO regulates the expression of genes involved in **apoptosis, cell cycle arrest and stress resistance**

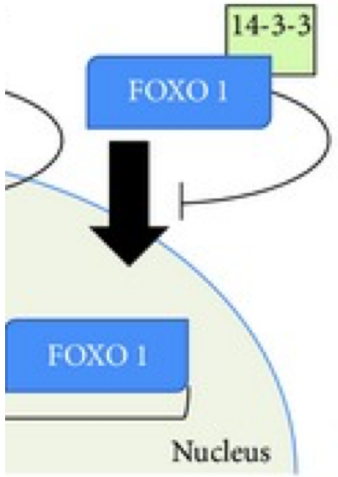
Akt phosphorylates GSK3 (Glycogen Synthase Kinase 3) and **inhibits** its functions

GSK3 represses several proteins (**NF-AT**) involved in **proliferation, migration, inflammation, glucose metabolism**

Akt and cell survival



1. Phosphorylates **FOXO,** thus blocking its nuclear translocation (sequestration by 14-3-3) and the expression of pro-apoptotic **BIM.**

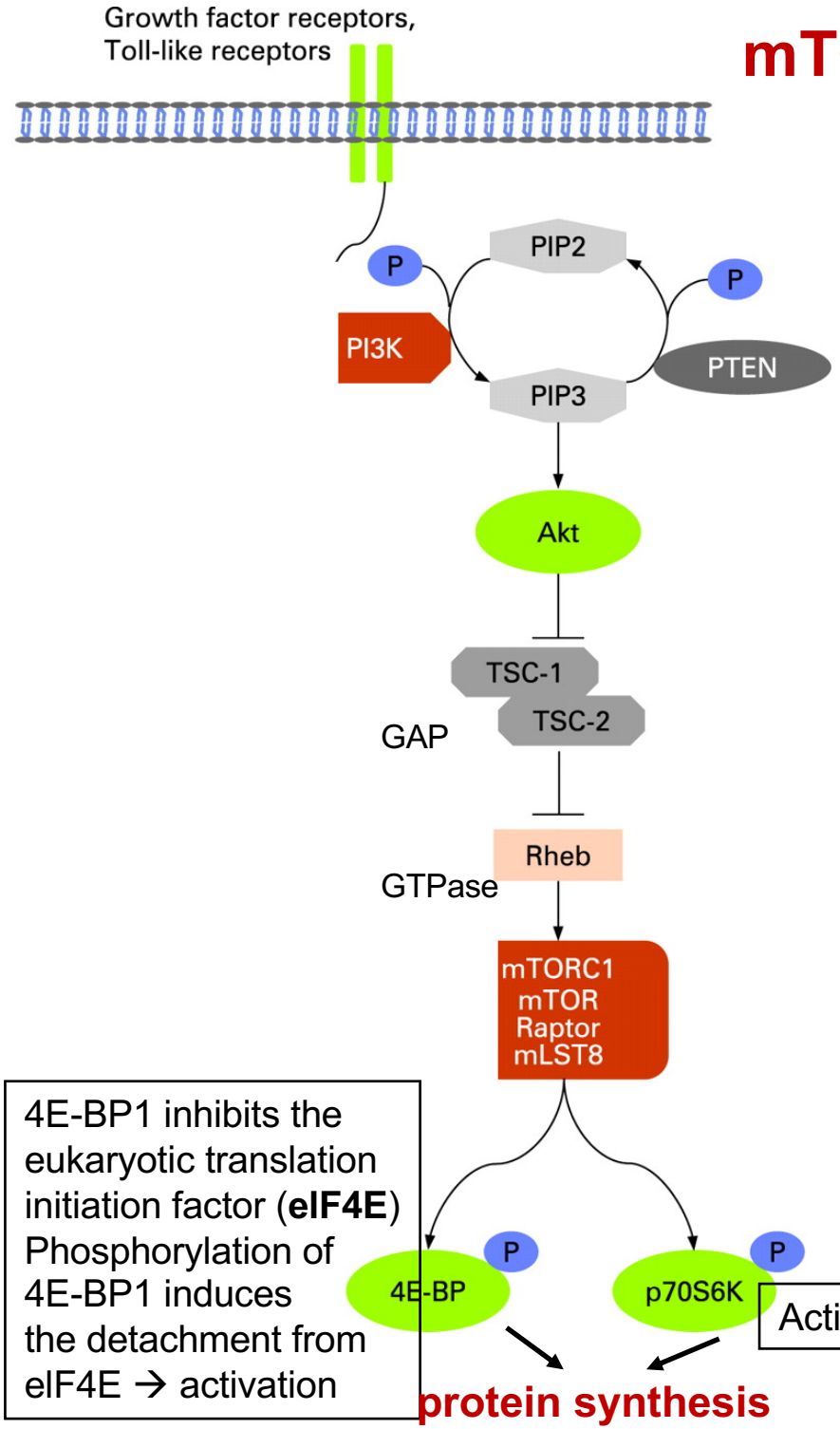


2. Phosphorylates and **inactivates pro-apoptotic BAX and BAD** (sequestration by 14-3-3).

3. Induces the expression of **anti-apoptotic Bcl-xL.**

4. Favors **Mdm2-mediated degradation of p53.**

mTOR (mechanistic or mammalian target of rapamycin)



Belongs to a family of **Ser/Thr kinase** referred as class IV PI3Ks

Crucial **regulator of metabolism**, cell **growth** and **proliferation** by monitoring nutrient availability, cellular energy levels, oxygen levels and mitogenic signals.

mTOR is part of two distinct complexes: **mTORC1** and **mTORC2**

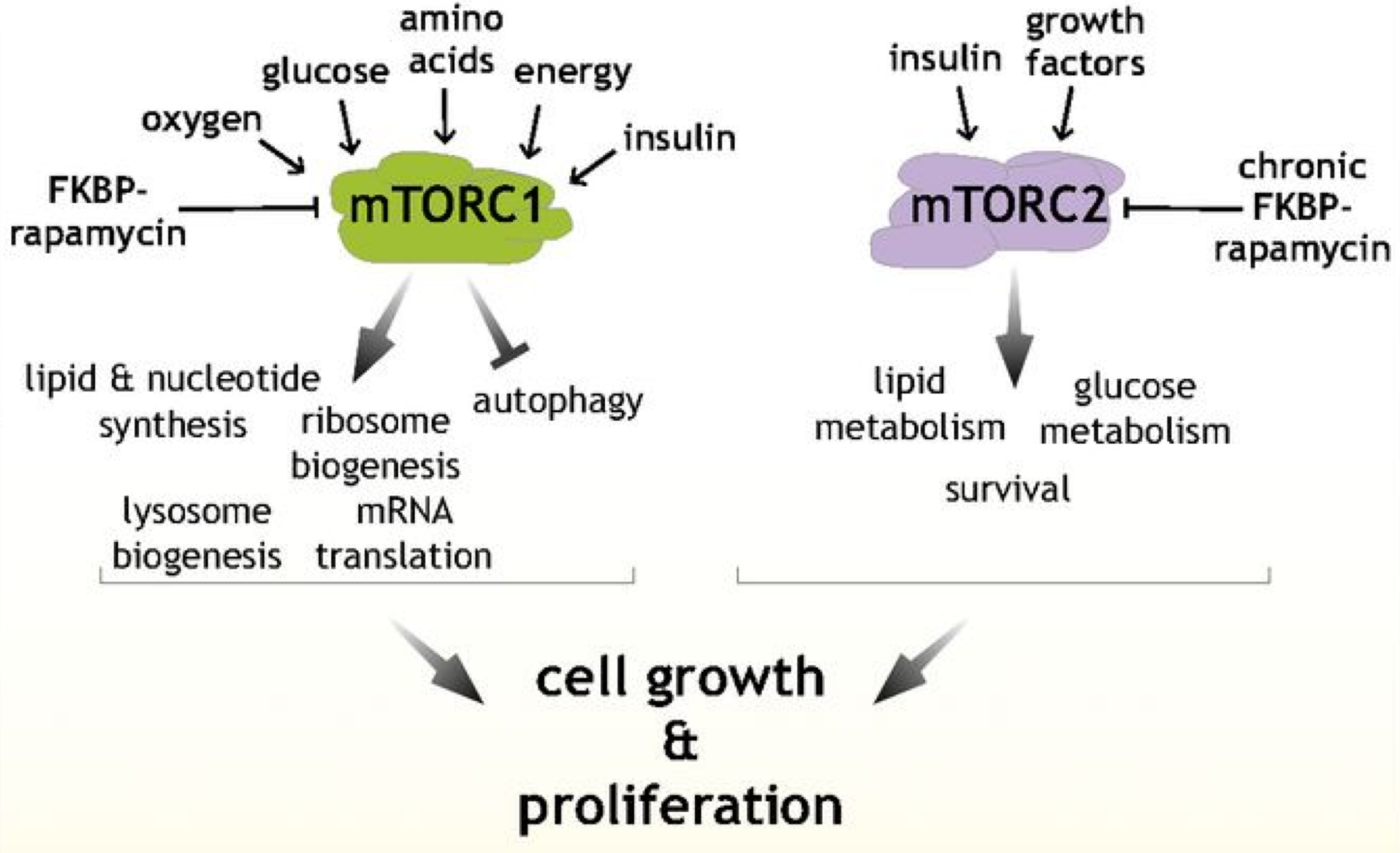
Akt activates mTORC1 by phosphorylating **TSC-1/TSC-2** complex **that blocks the mTORC1** complex

mTOR phosphorylates and activates the ribosomal protein S6 kinase 1 (**p70S6K1**) and eucaryotic translation initiation factor 4B (eIF4E)-binding protein (**4EBP1**) → **promotion of protein synthesis.**

4E-BP1 inhibits the eukaryotic translation initiation factor (**eIF4E**) Phosphorylation of 4E-BP1 induces the detachment from eIF4E → activation

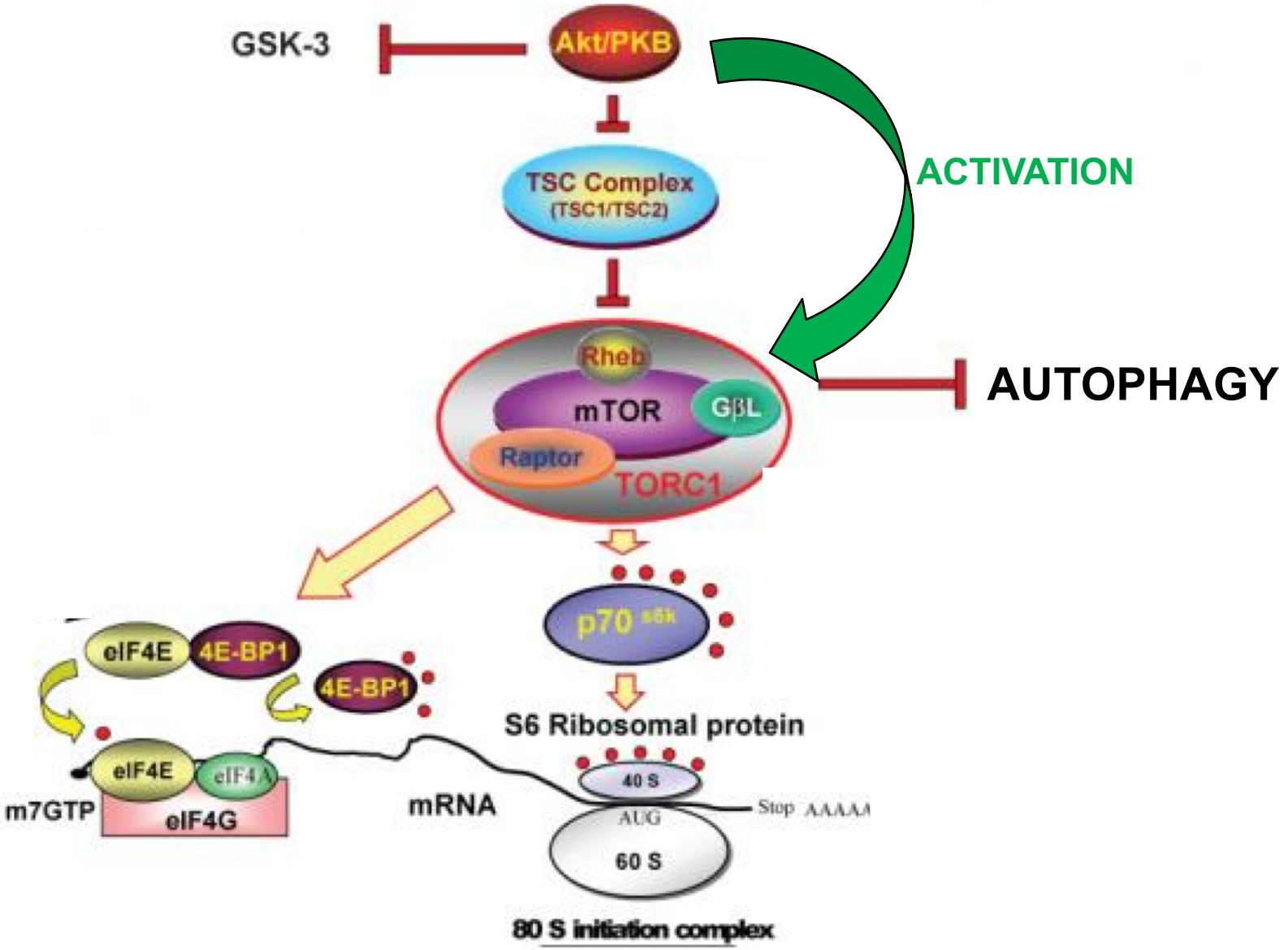
Activation of ribosomal protein S6 kinase 1

mTOR: the catalytic subunit of mTORC1 and mTORC2 complexes

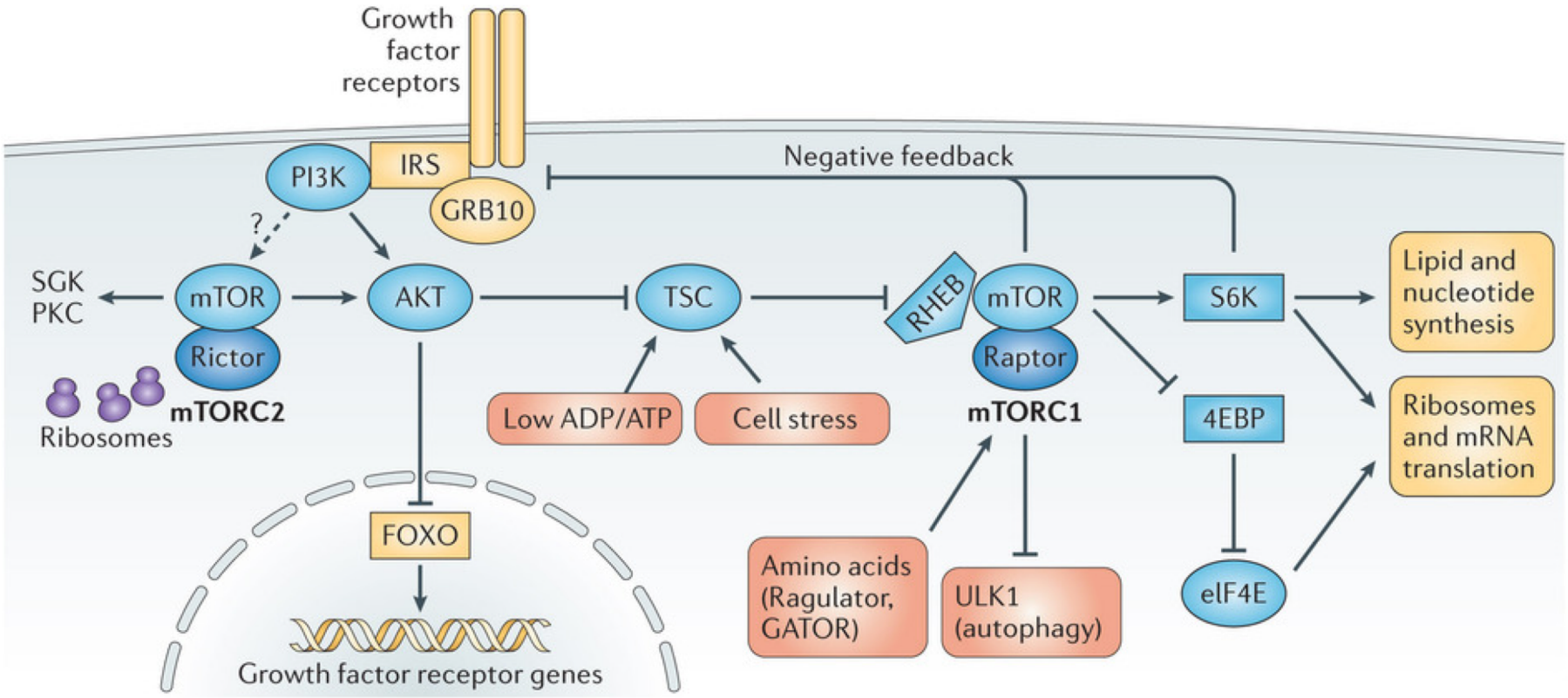


Schematic showing the signals sensed by mTORC1 and mTORC2 and the processes they regulate to control growth.

Functions of mTORC1



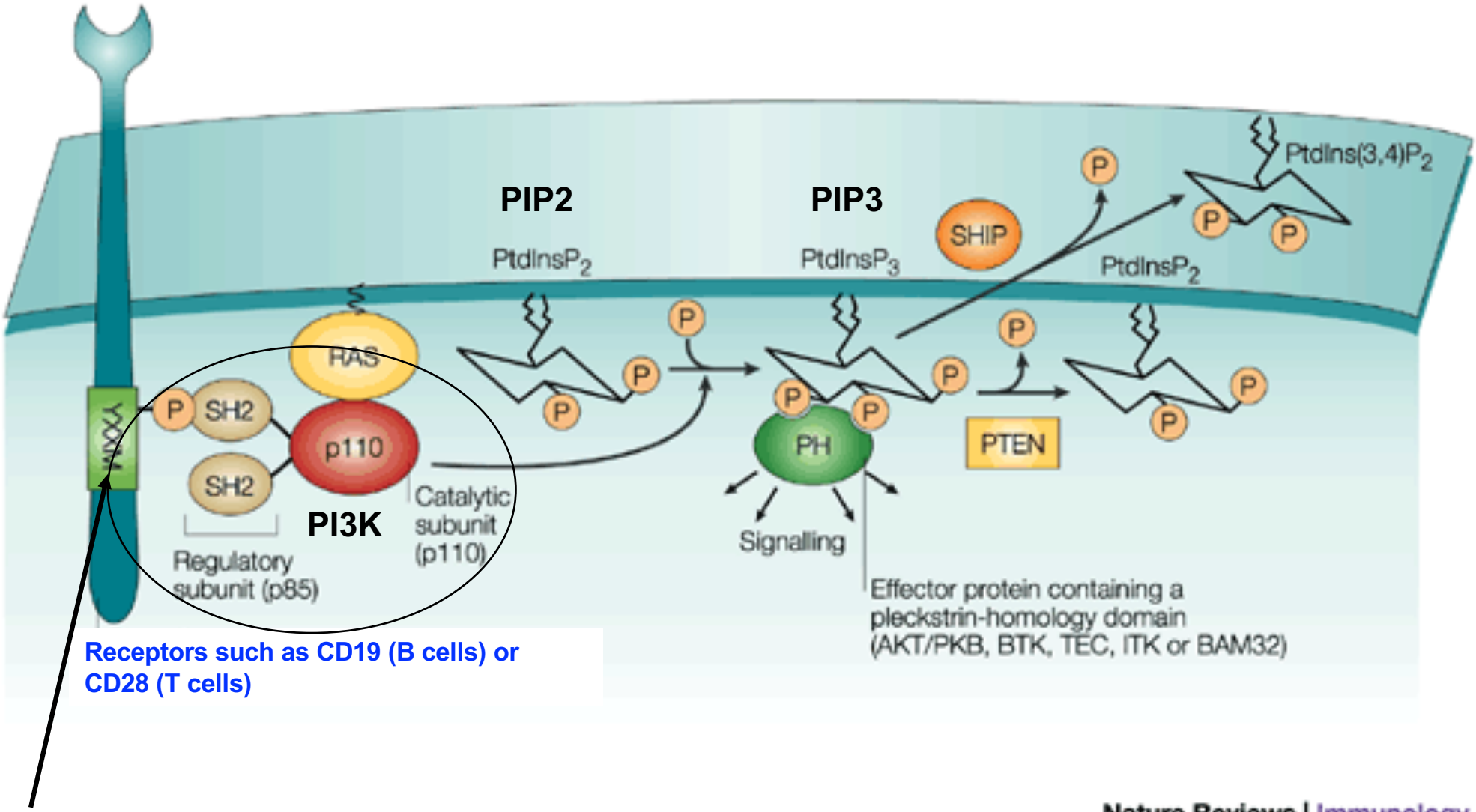
Functional effects of PI3K/Akt/mTOR



Akt phosphorylates and inhibits glycogen synthase kinase 3 (**GSK3**) and forkhead box family of transcription factors (**FOXO**) → **cell survival, proliferation** and **metabolism genes**

mTORC1 promotes anabolic processes: mRNA synthesis, **ribosome biogenesis** (protein synthesis), **synthesis of lipids** and **nucleotides**

CLASS 1A PI3K signaling pathway in lymphocytes



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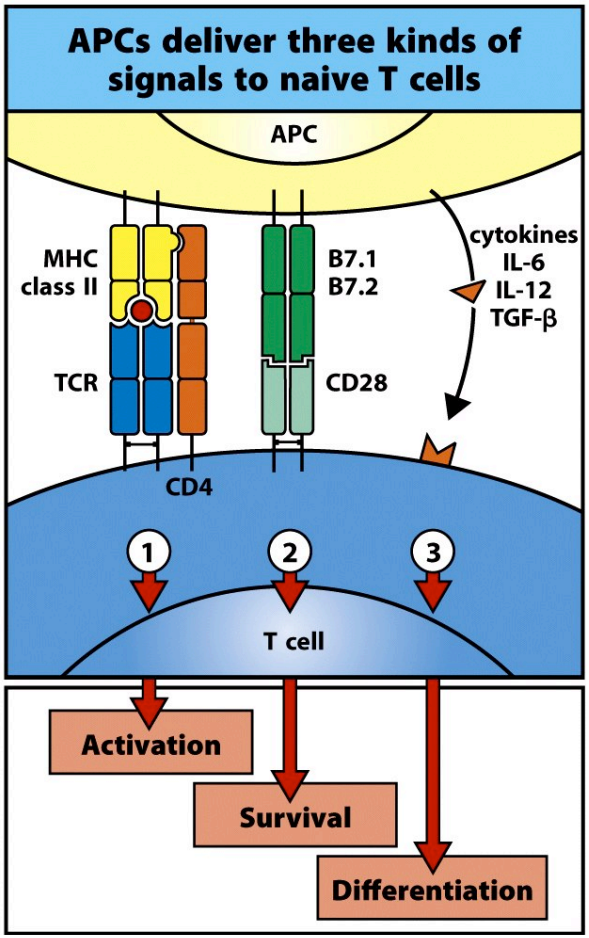
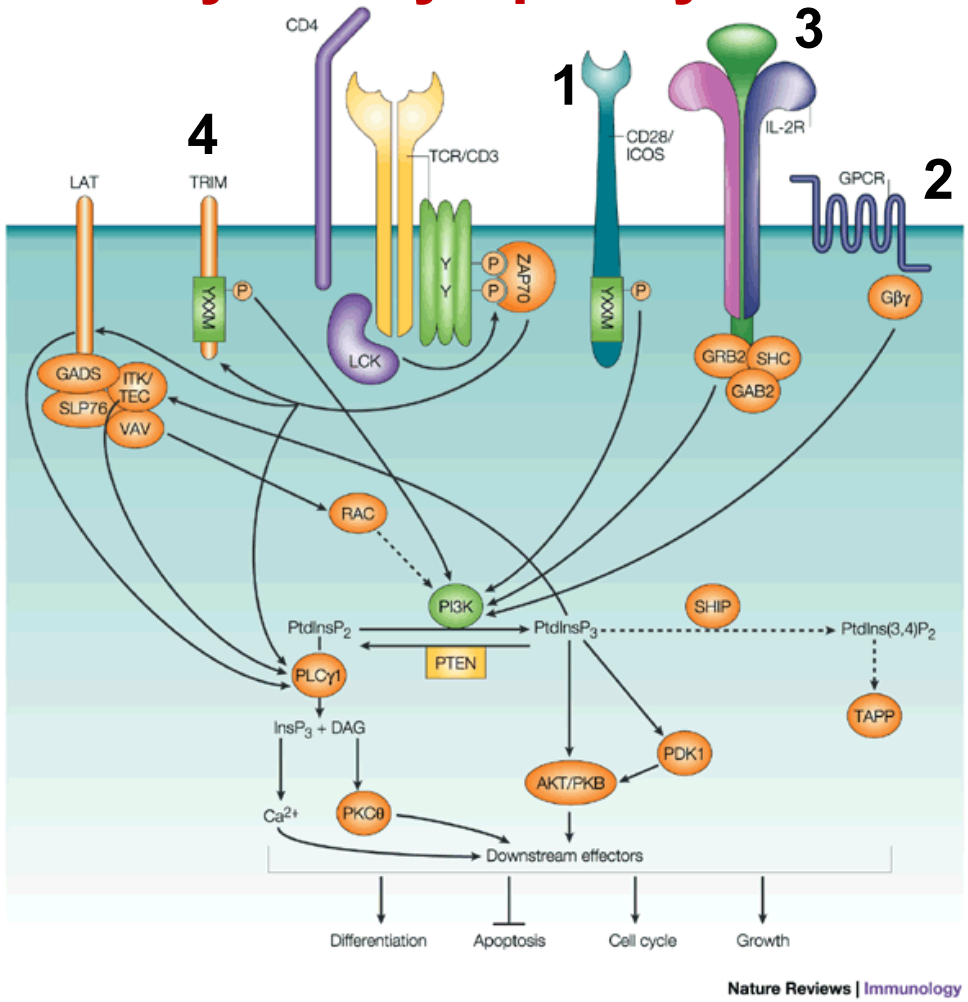
^{P}YXXM: p85 binding motif

PI3K signaling pathway in T lymphocytes





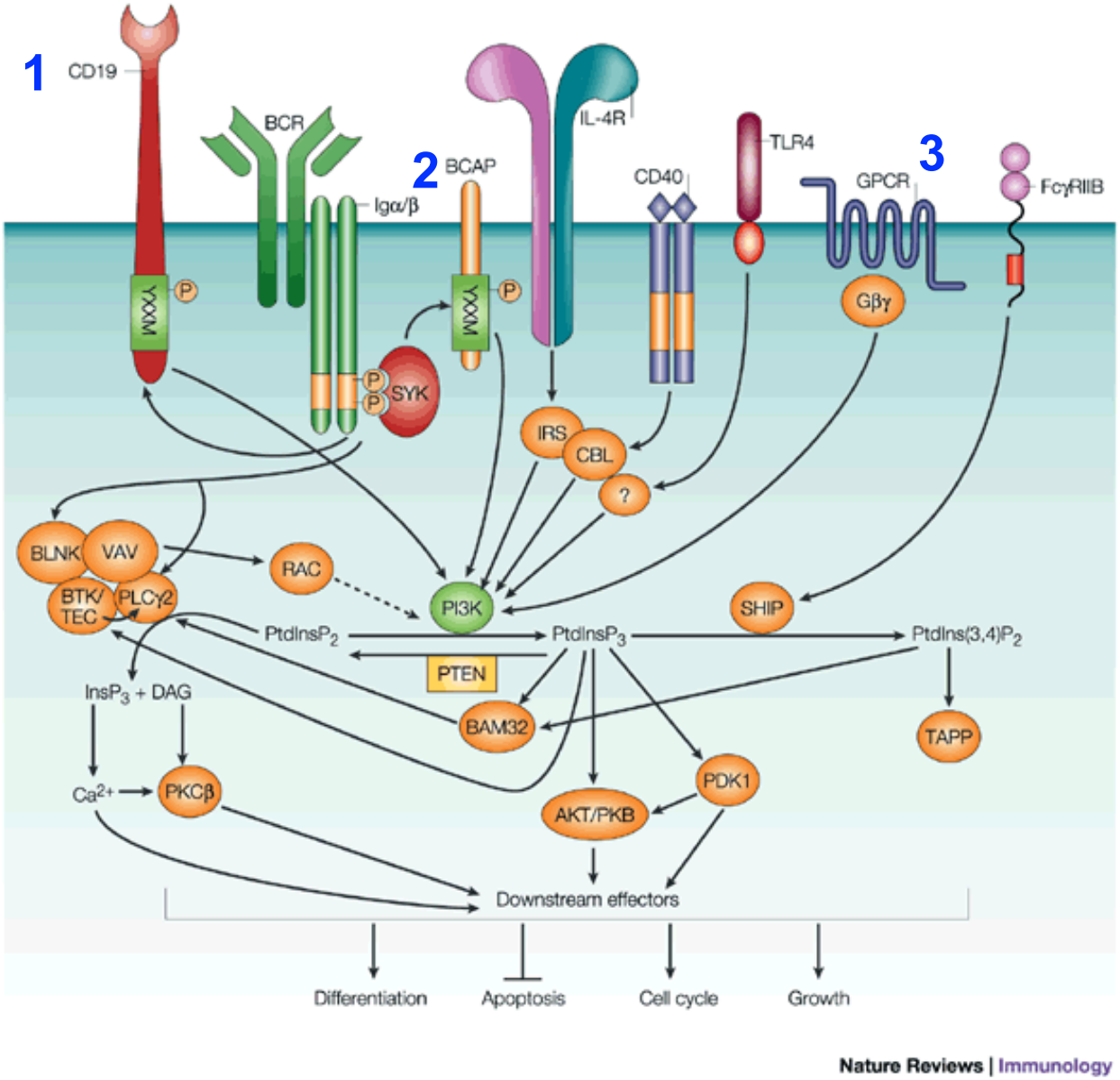
1 **CD28 (Costimulatory molecule)** intracytoplasmic tails contain the **YxxM** sequence that is phosphorylated following CD28 interaction with B7.1 or B7.2 expressed on APCs and binds the SH2 domains of **p85 subunit of class 1A PI3K**

2 **Chemokine receptors** activate both **class 1A and class 1B PI3K**

3 **IL-2R** activates both **class 1A and 1B PI3K**

4 **TRIM (adaptor molecule)** is a palmitoylated protein present in lipid rafts that contains a **YXXM** that is phosphorylated following TCR stimulation and recruits **class 1A PI3K**

PI3K signaling pathway in B lymphocytes



1. Costimulatory molecules:

CD19 intracytoplasmic tail contains the **YxxM** sequence that is phosphorylated following stimulation and binds the SH2 domains of **p85 subunit of class 1A PI3K**

2. Adaptor molecules:

BCAP is a palmitoylated protein present in lipid rafts that contains a **YXXM** that is phosphorylated following BCR stimulation and recruits **class 1A PI3K**

3. Chemokine receptors

activate both **class 1A and class 1B PI3K**

FcγRIIB blocks PI3K pathway by recruiting **SHIP** a phosphatase that dephosphorylates **PI (3,4,5)P3 in position 5** and generate PI(3,4)P2