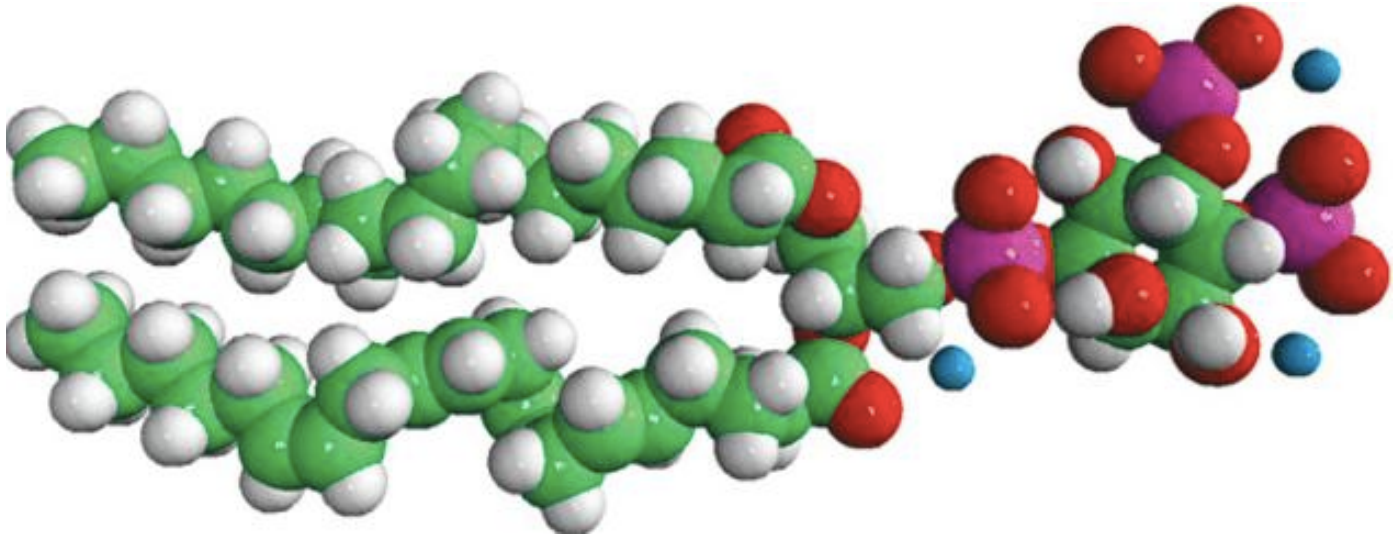
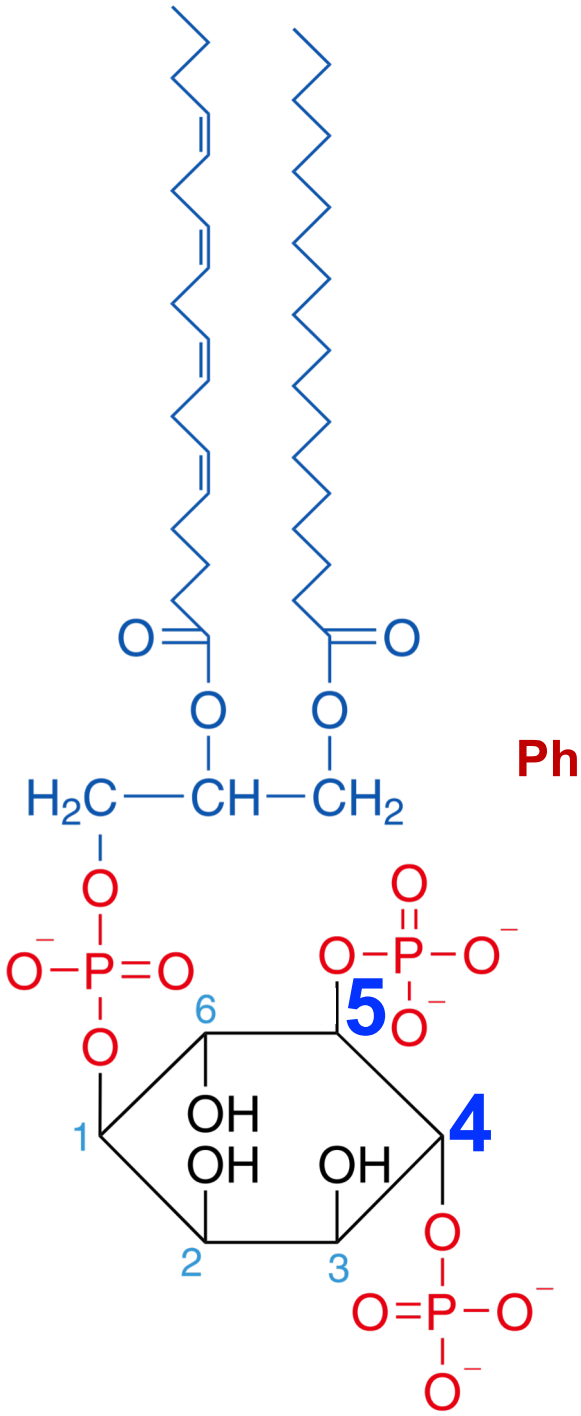


I fosfoinositidi nella segnalazione delle cellule del sistema immunitario



PIP2

Phosphatidylinositol 4,5-bisphosphate

Directional Neutrophil migration

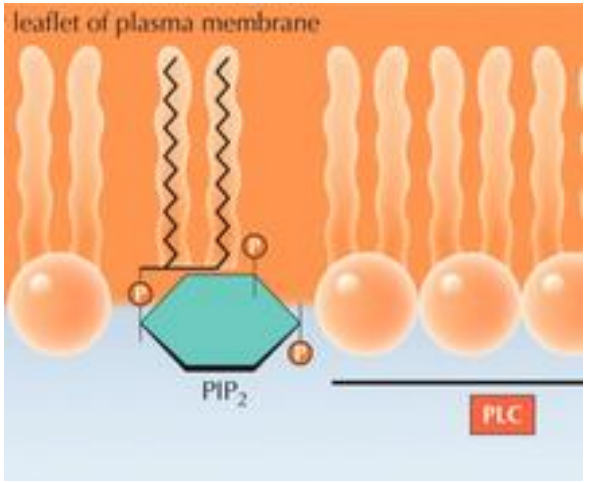
Macrophage phagocytosis

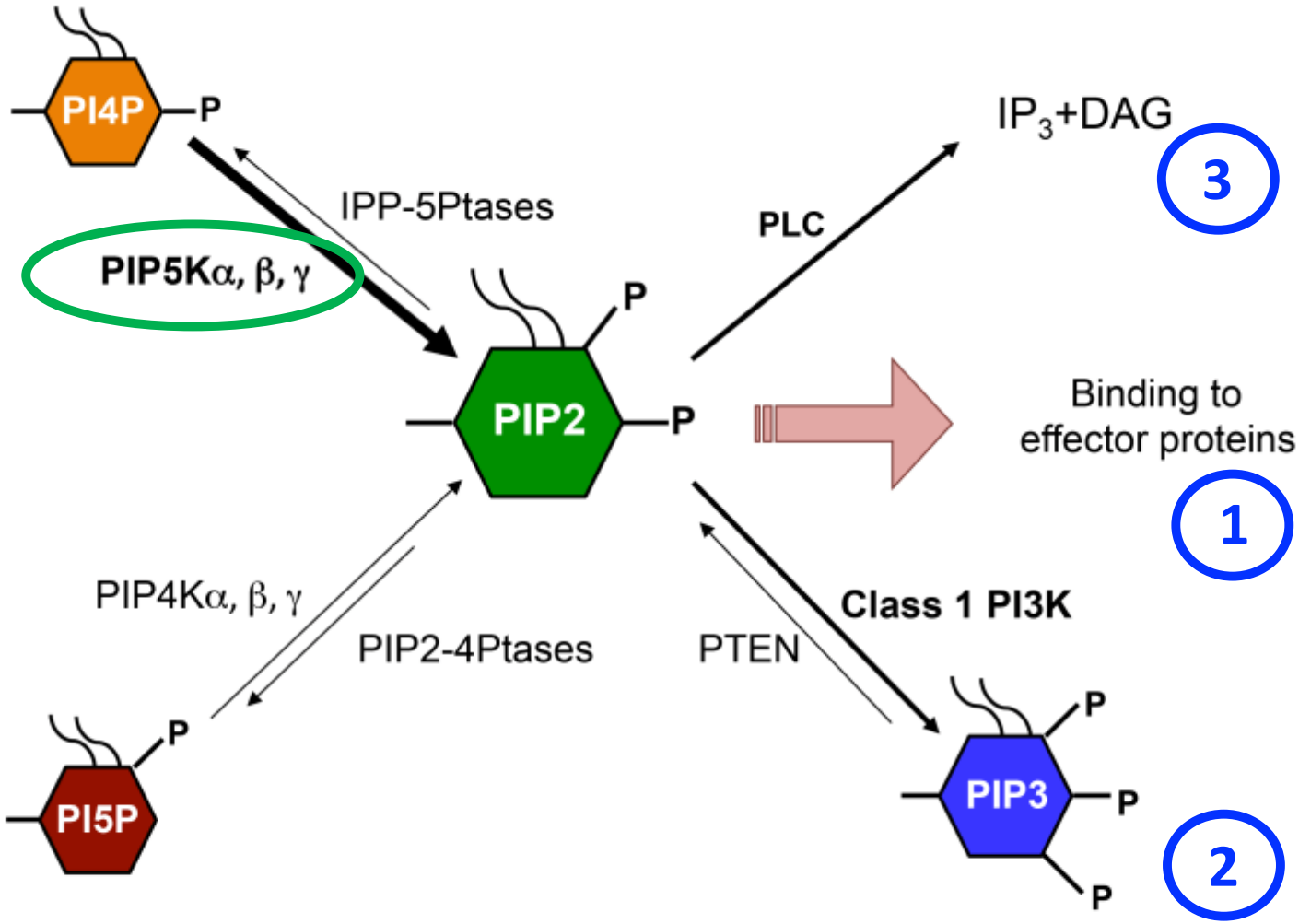
Calcium signals and gene transcription in lymphocytes, NK cells and mast cells

Integrin-dependent adhesion of T cells

Lysosome secretion and trafficking at immune synapse in cytolytic and secretory cells

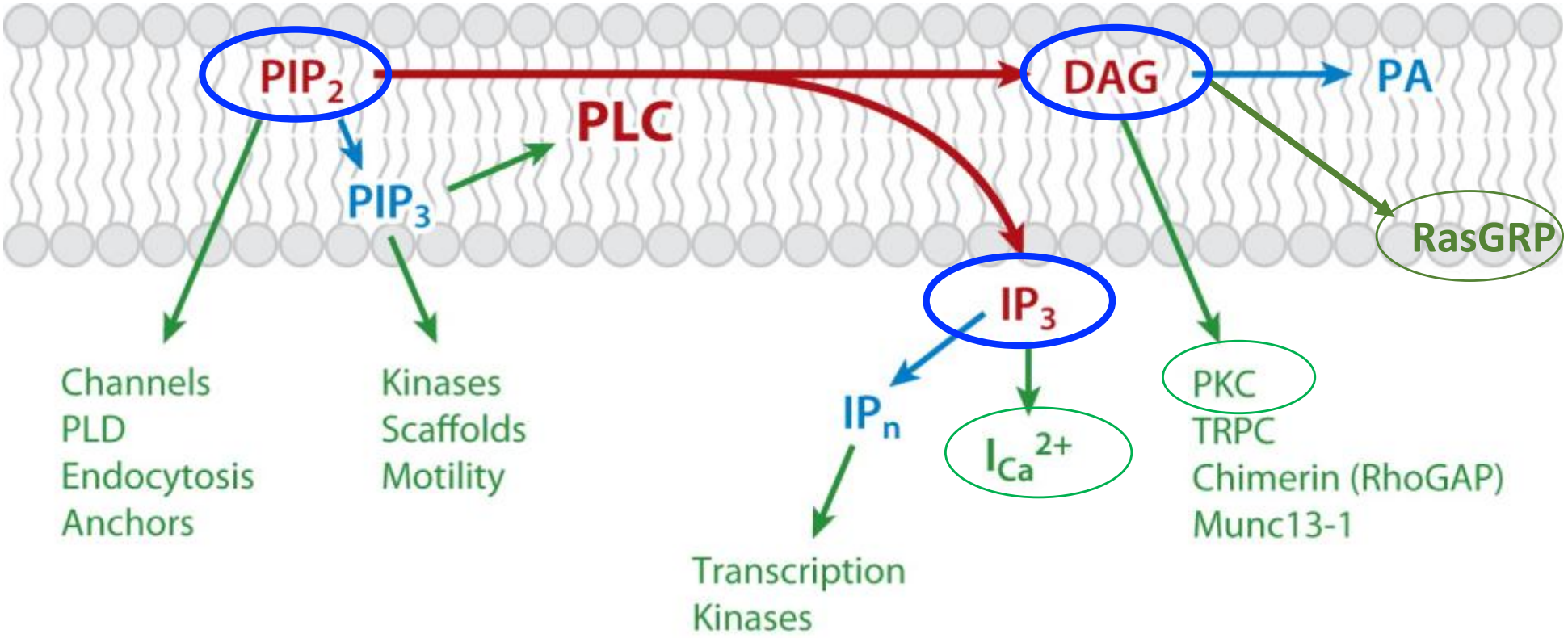
PIP2 synthesis and turnover

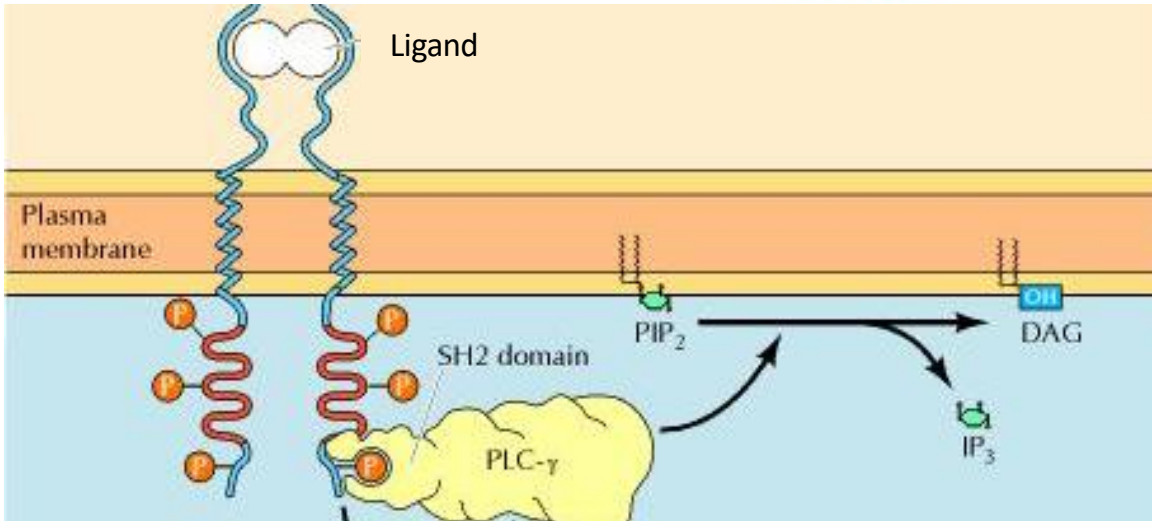




- 1% membrane phospholipids → neo-synthesis for ensuring downstream signalling functions.
- PIP2 is mainly synthesized by **PIP5K** that phosphorylate PI4P and to a lesser extent by PIP4K that phosphorylate PI5P.
- PIP2 may also derive from PTEN-mediated PIP3 dephosphorylation.

Phosphatidylinositol 4,5-bisphosphate (PIP2)

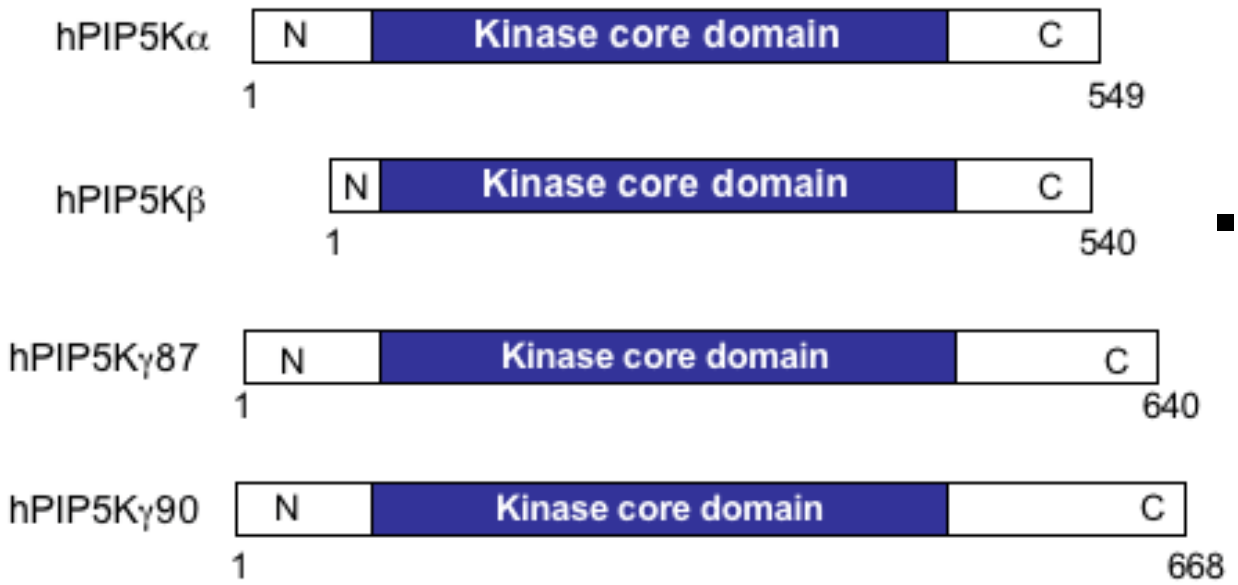




PLC = fosfolipasi C

DAG = diacilglicerolo
IP3 = Inositolo 1,4,5-trifosfatato

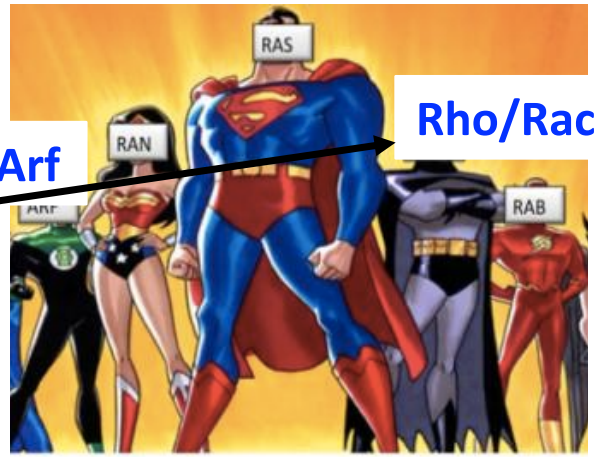
(Phosphatidylinositol-4-phosphate 5-kinase) PIP5K family

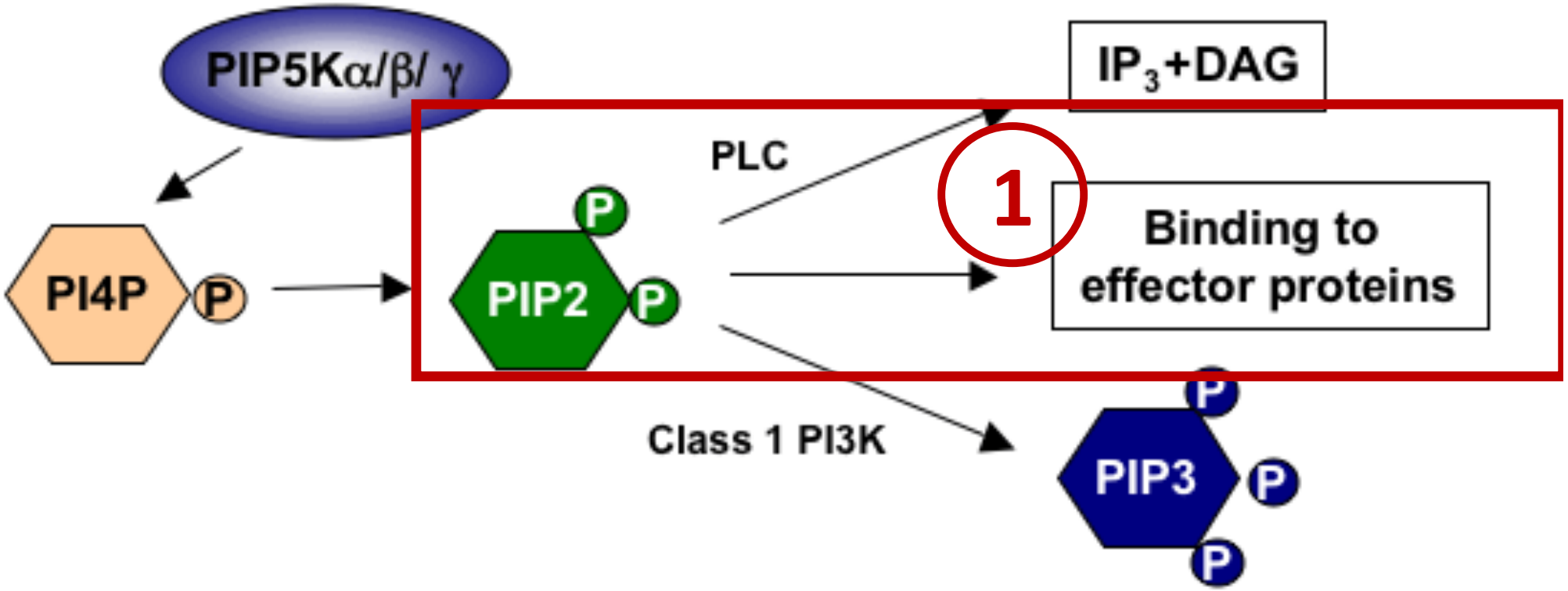


- Tre isoforme: PIP5Kα; PIP5Kβ and PIP5Kγ

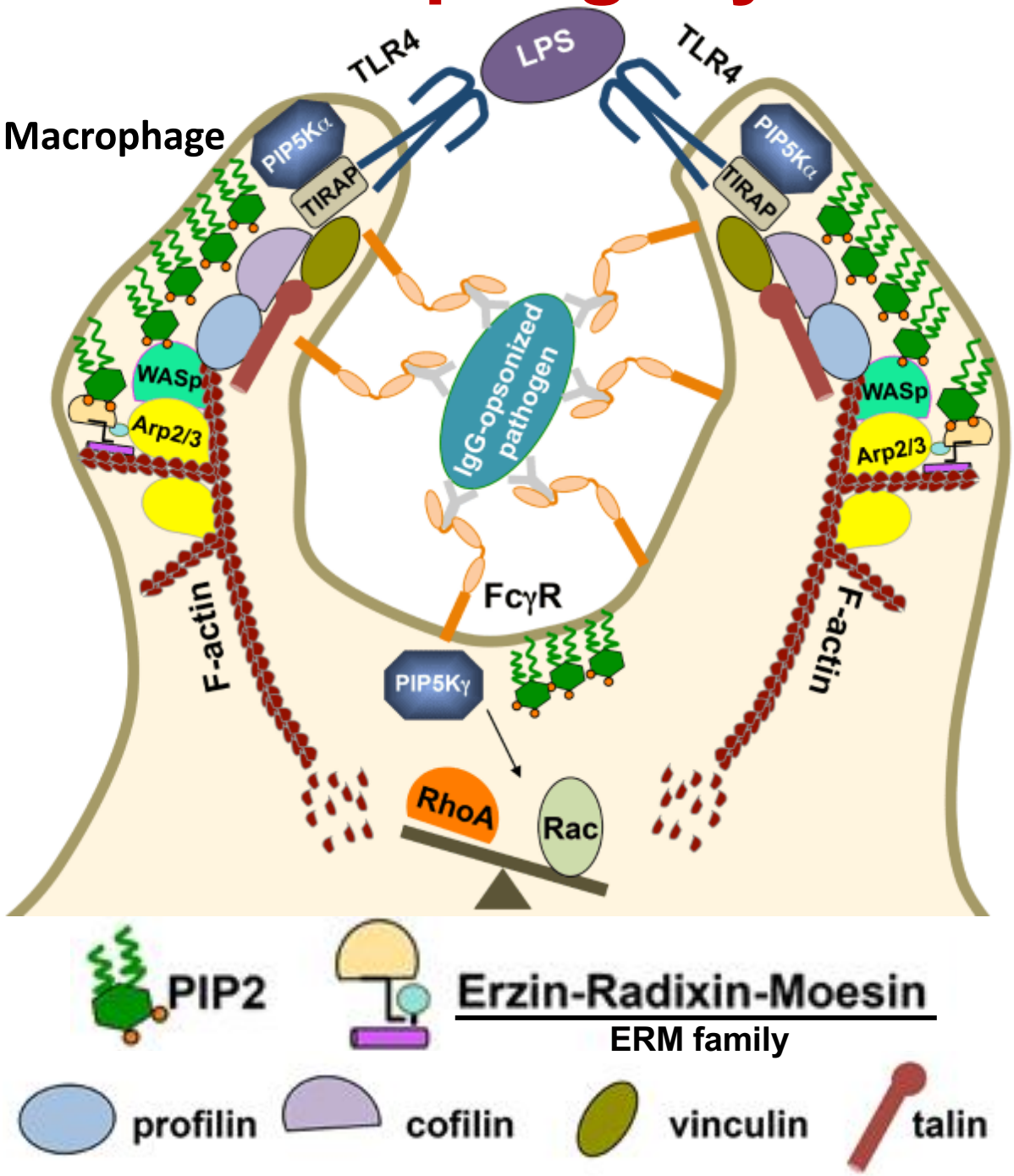
- Nell'uomo sono presenti: tre varianti di splicing per PIP5Kα, quattro per β e tre per γ.
- Tutte le isoforme e varianti di splicing di PIP5K condividono una significativa omologia di sequenza nel dominio catalitico

Regolazione e attivazione di PIP5K dipendono da alcuni membri della famiglia delle ''small GTPasi''




PIP5Kα/β/ γ
PI4P
P
PIP2
P
P
PLC
IP3+DAG
1
Binding to
effector proteins
Class 1 PI3K
PIP3
P
P
P

PIP2 in phagocytosis



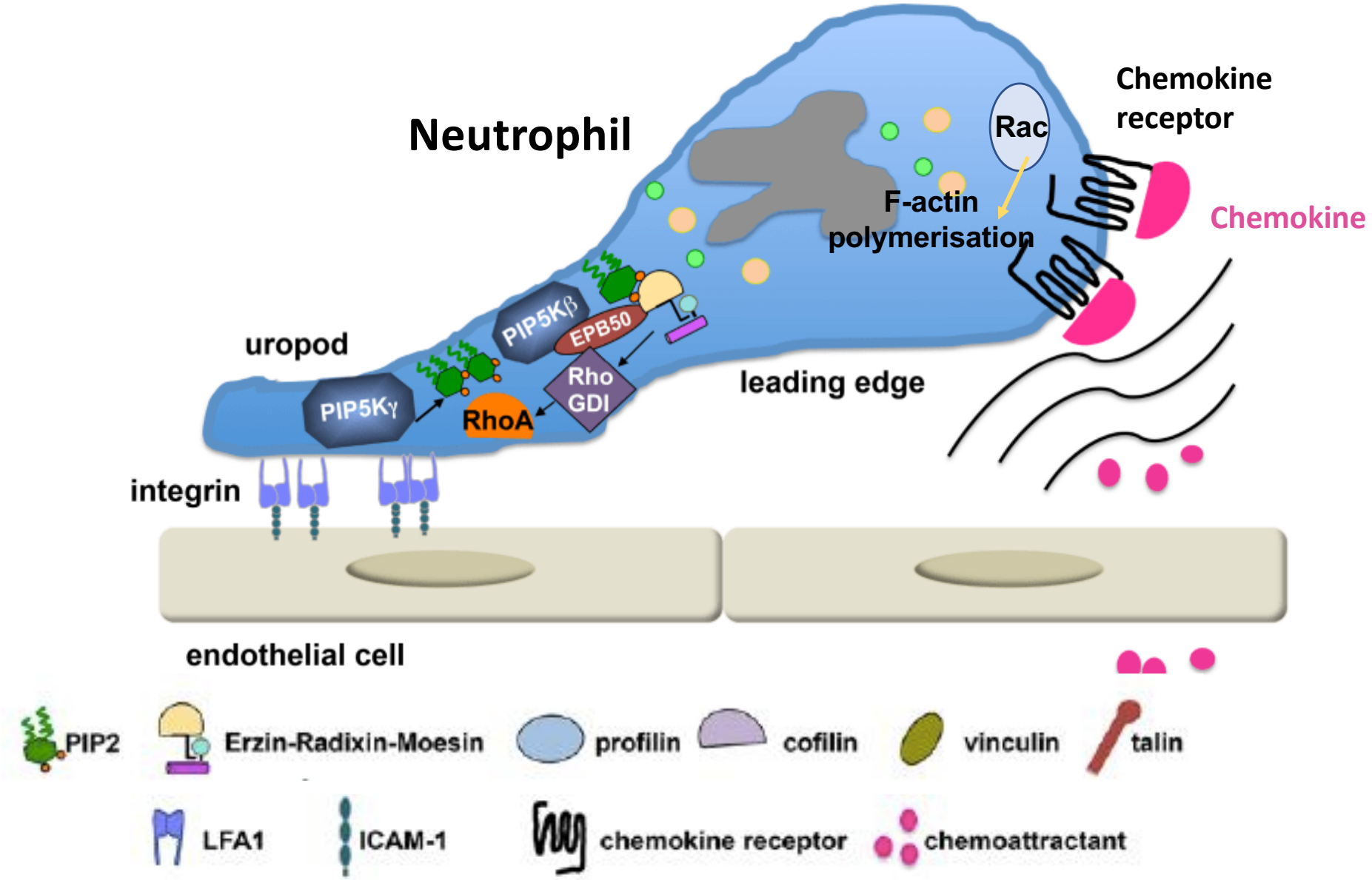
During phagocytosis PIP2 is crucial for assembly and remodelling of F-actin structure. It accumulates in the inner leaflet of phagosomal cup and recruits/activates actin polymerization proteins (profilin, cofilin, talin, vinculin, WASP, erzin-radixin-moesin family members, etc).

PIP5Kα is mainly recruited and activated by plasma membrane **TLR** (i.e. TLR4)

PIP5Kγ is recruited to **FcγR**

Both isoforms are involved in the reorganization of actin cytoskeleton required for phagocytosis and signalling

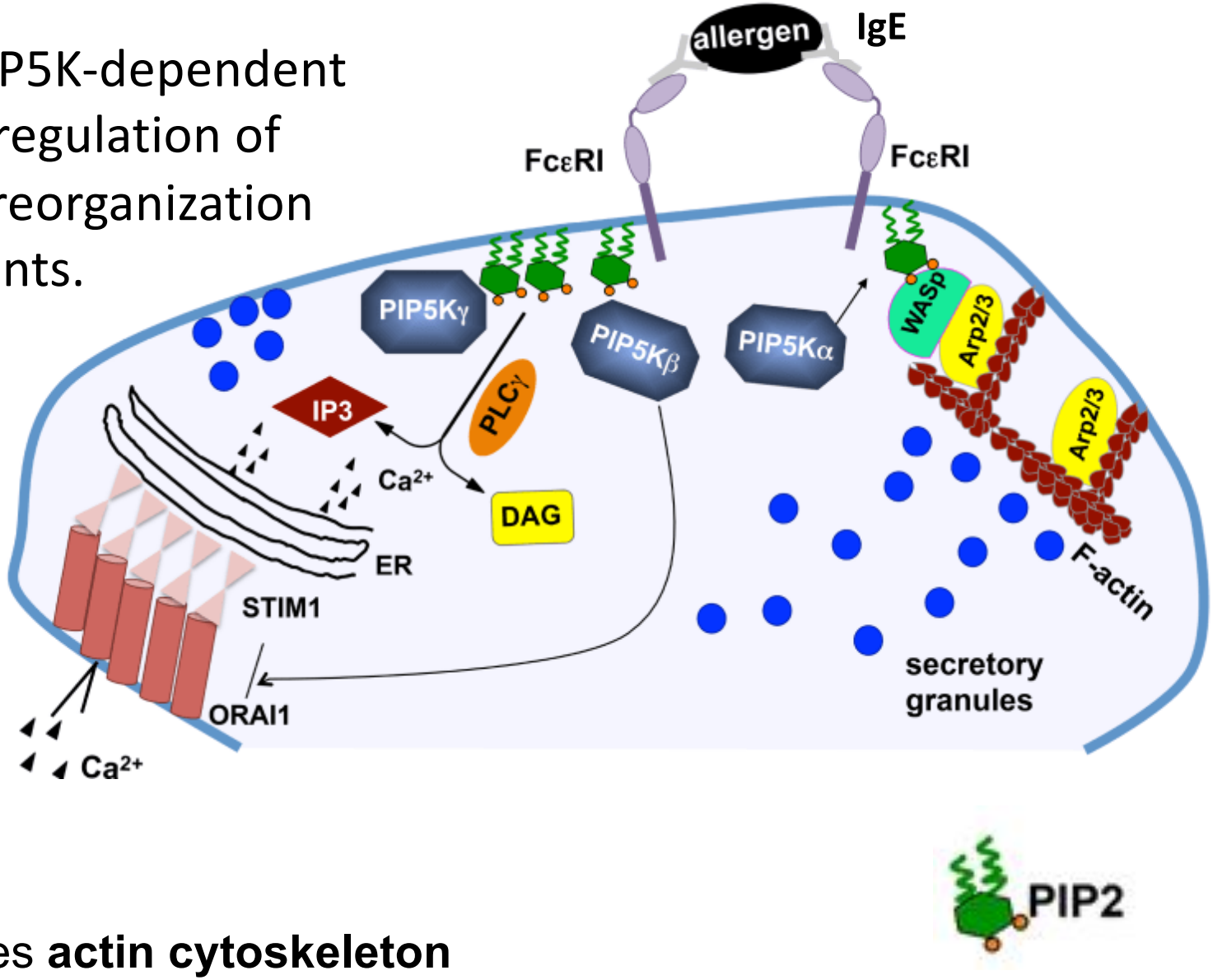
PIP2 chemotaxis and adhesion



PIP5Kβ and **PIP5Kγ** isoforms control signals involved in cell polarization during cell migration, chemotaxis and adhesion to endothelial cells.

PIP2 in mast cells

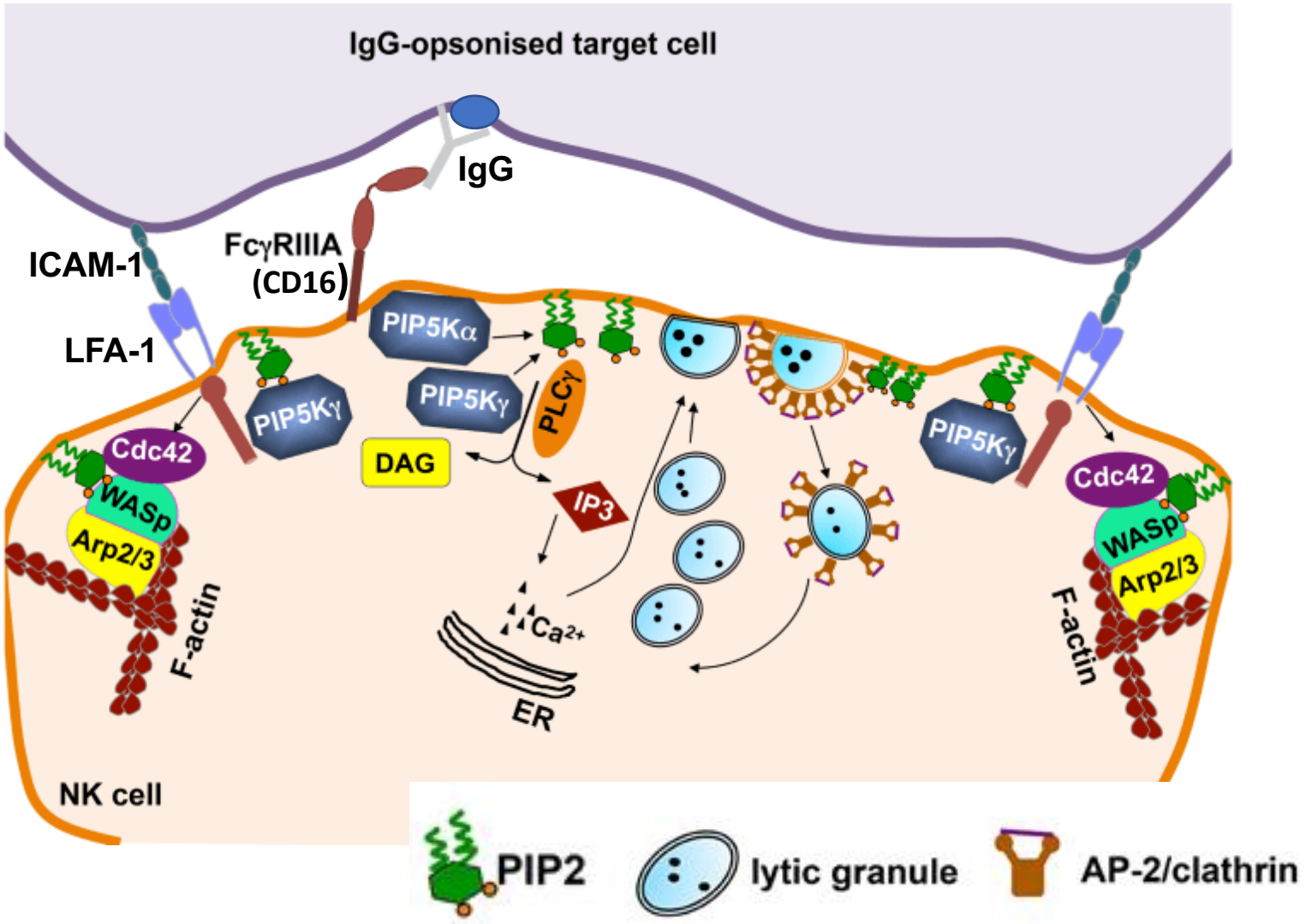
Involvement of PIP5K-dependent PIP2 pools in the regulation of the cytoskeleton reorganization and signalling events.



PIP5Kα regulates **actin cytoskeleton**
PIP5K β and γ isoforms regulates **FcεRI-induced Ca2+** response and granule release.

PIP2 in Natural Killer cells

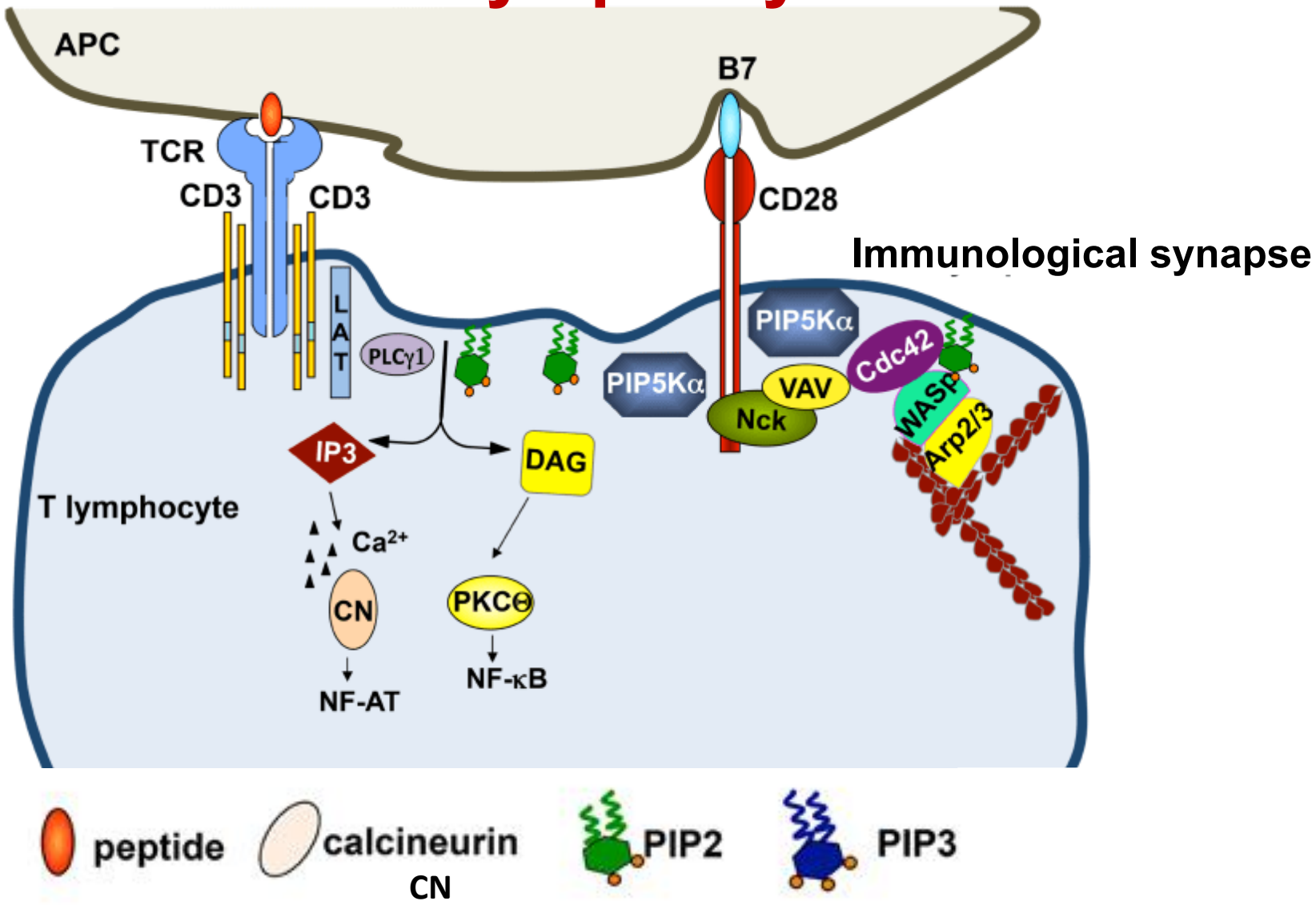
NK cells contribute to immune defence through their effector functions: cytotoxicity and cytokine secretion



PIP5Kα and γ isoforms control **FcγRIIIA(CD16)-dependent Ca2+ response** and lytic **granule exocytosis**.

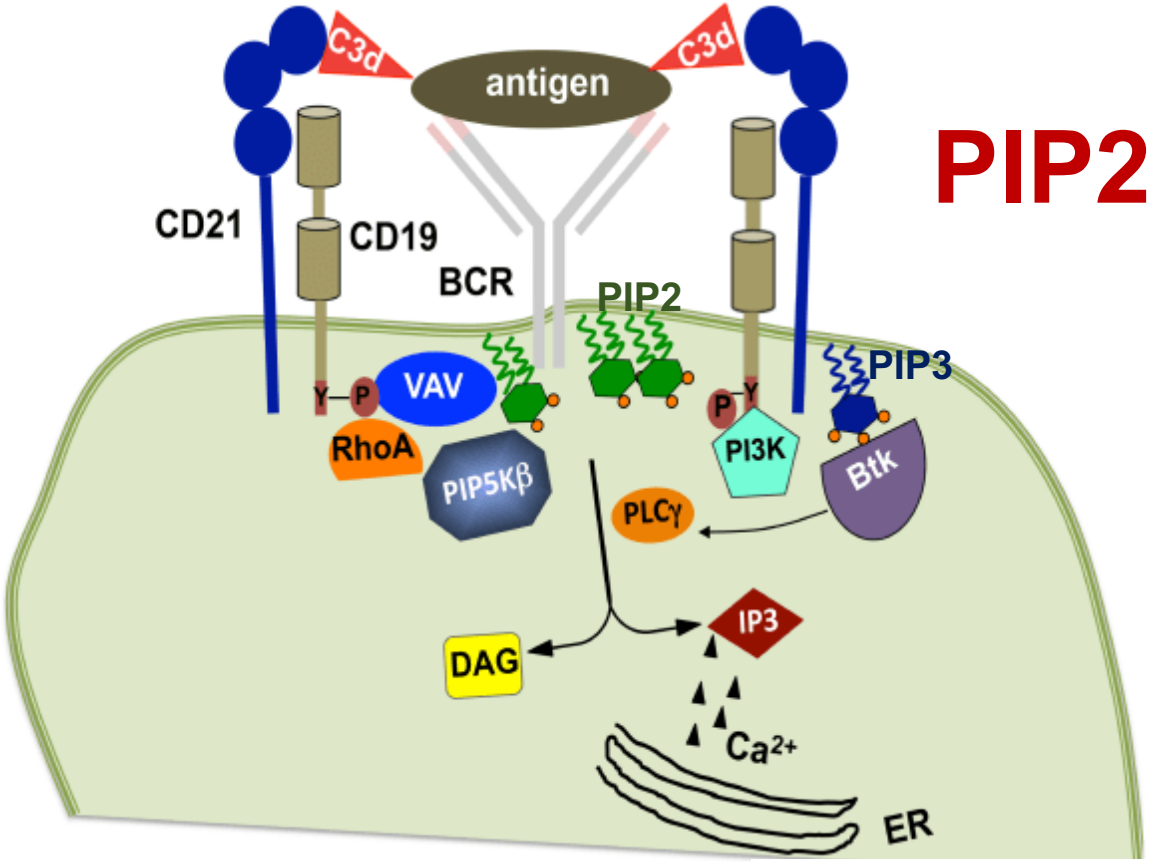
PIP5Kγ also regulates **integrin-mediated adhesion** and **recycling of lytic granule** components.

PIP2 in T lymphocytes



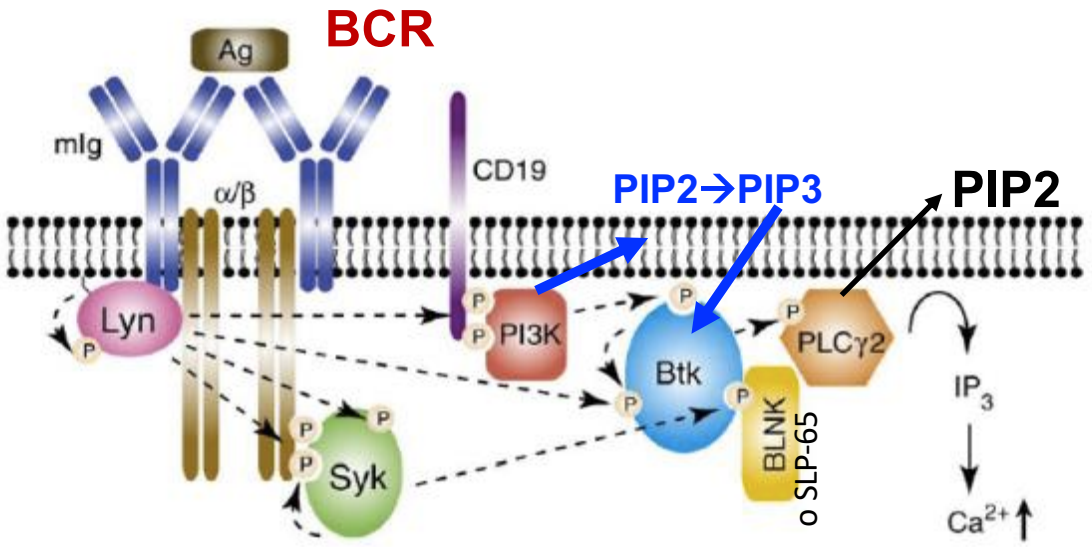
PIP2 is pivotal for T cell activation, serving as a substrate for both **PLCγ1** and **PI3K**
CD28 recruits PIP5Kα at the T:APC interface. **PIP5Kα**-dependent PIP2 pool is substrate of PLCγ1 for the generation of second messengers and **actin cytoskeleton reorganization.**

PIP2 in B lymphocytes

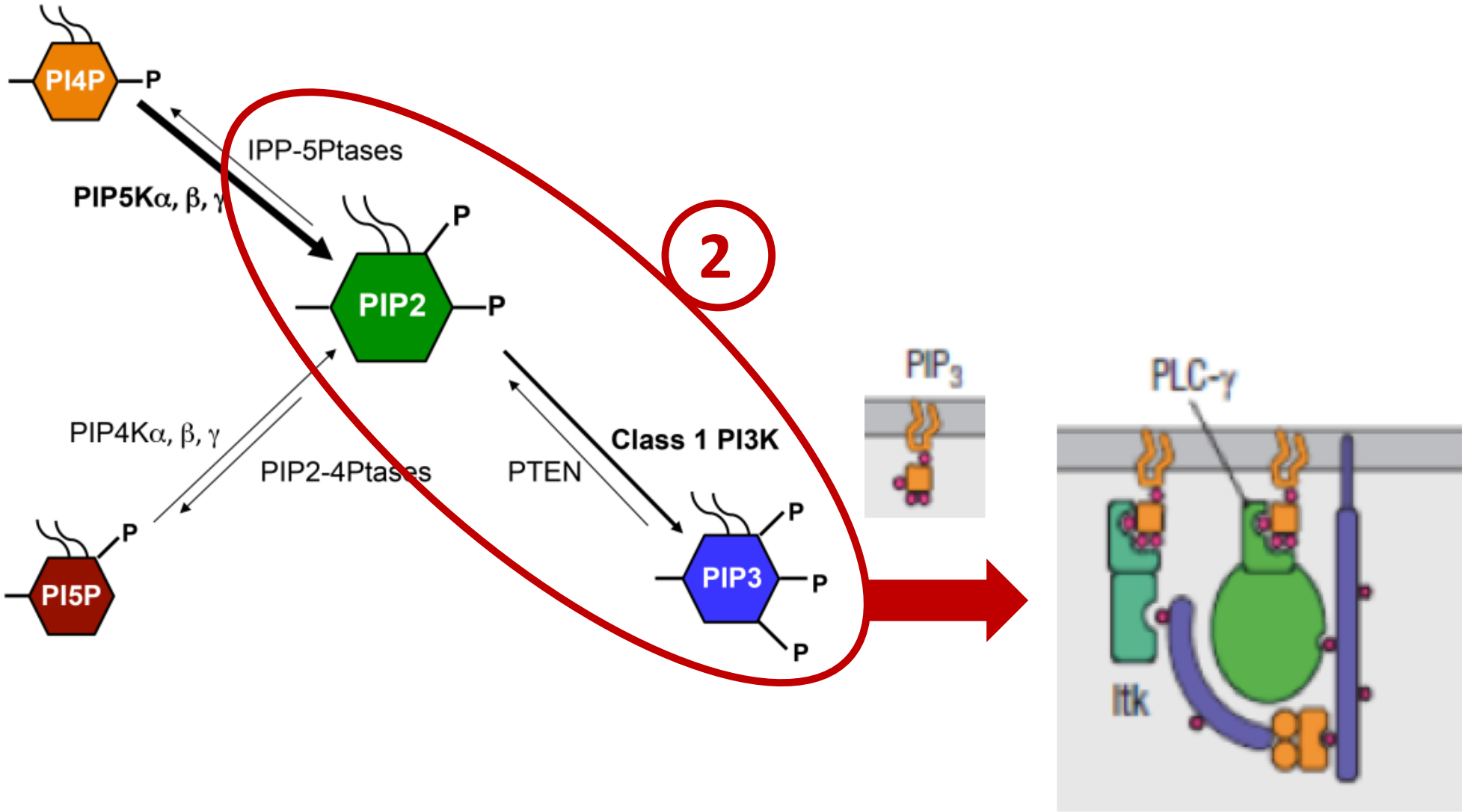


PIP2 is pivotal for B cell activation serving as a substrate for both **PLCγ2** and **PI3K**

CD19 recruits PIP5Kβ that increases the local levels of PIP2, thus, favouring **Btk-dependent PLCγ2** signalling pathways

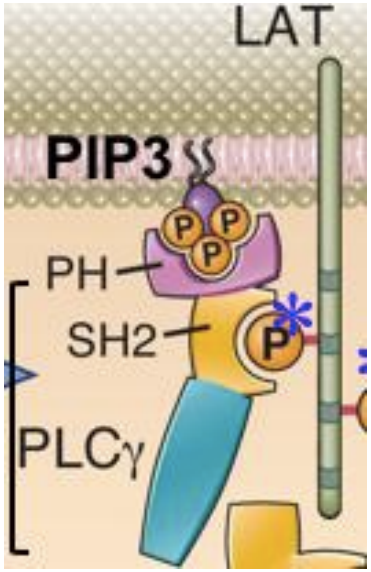


PIP2 è il substrato della PI3K che genera PIP3



Ruolo di PIP3 nel reclutamento della PLCγ

Phospholipase C subfamilies



PH domains bind **PIP3**

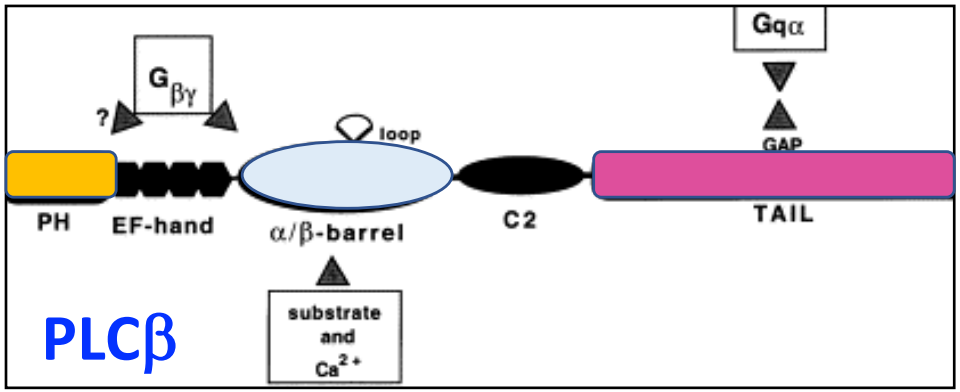
C2 domain binds Ca++ favouring the interaction with the membrane

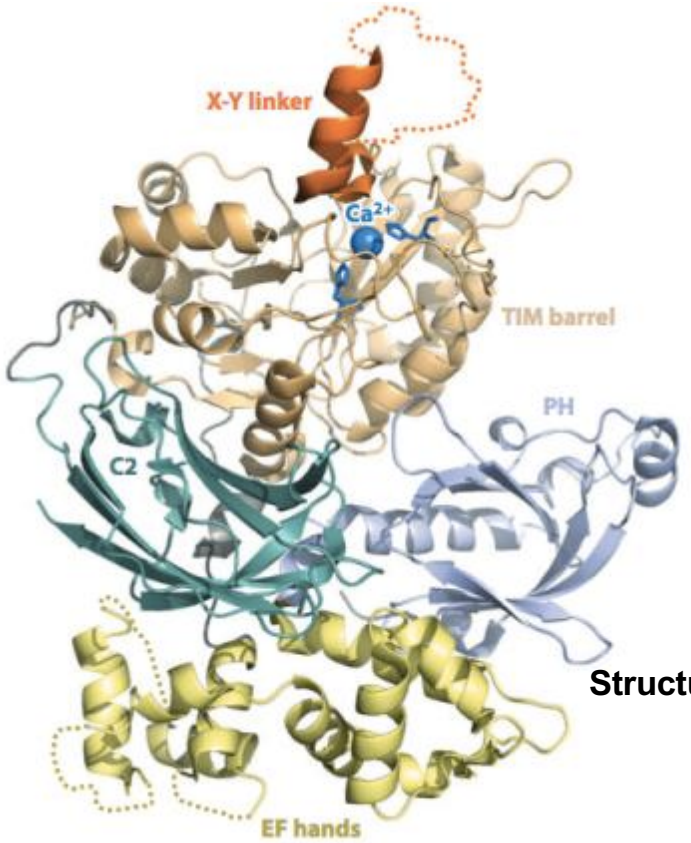
X-Y autoinhibitory linker

Kadamur G, Ross EM. 2013.
Annu. Rev. Physiol. 75:127–54

PLC subfamilies: different modes of activation

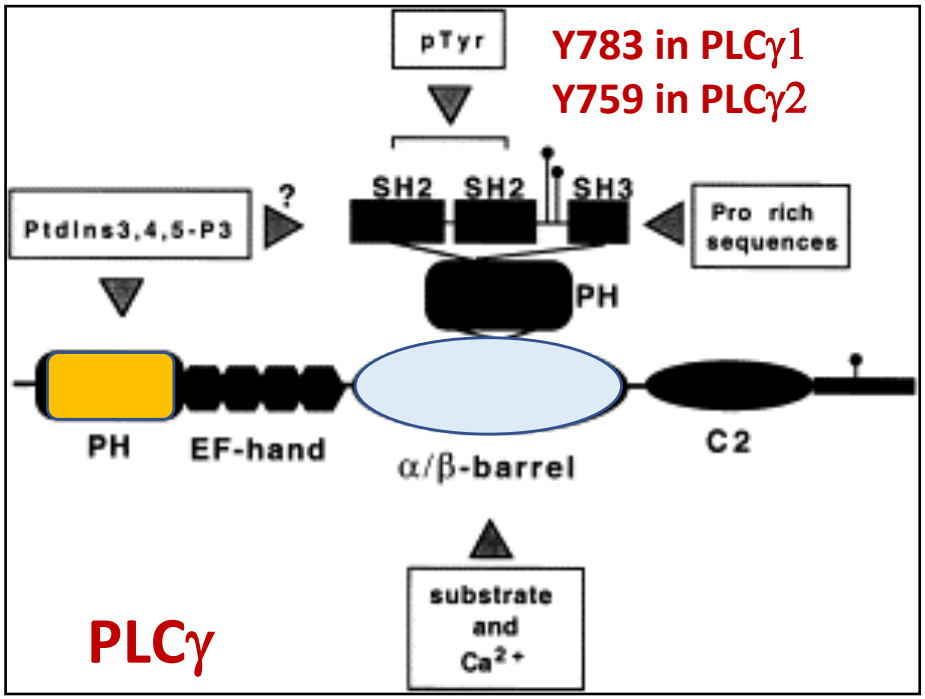
Heterotrimeric G proteins mediate activation of PLCβ



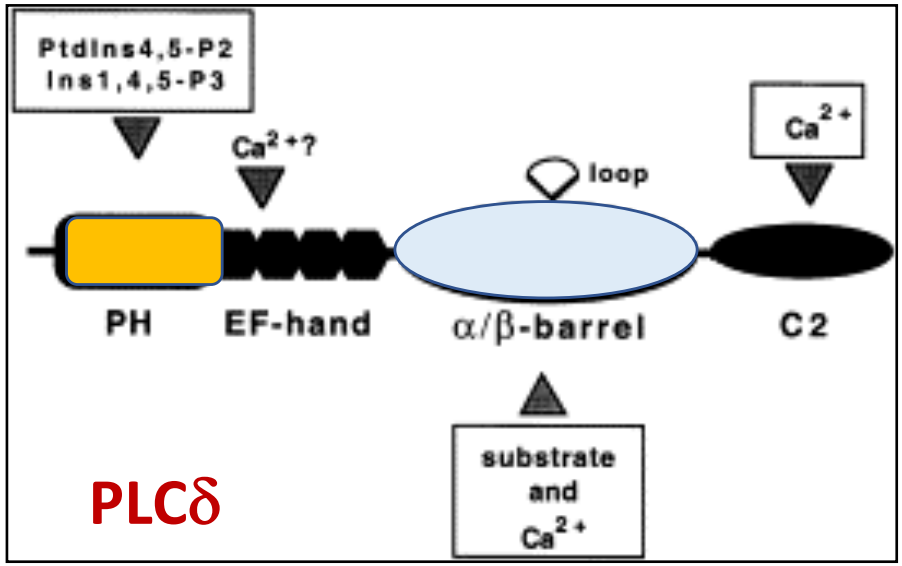


Structure of PLCβ2

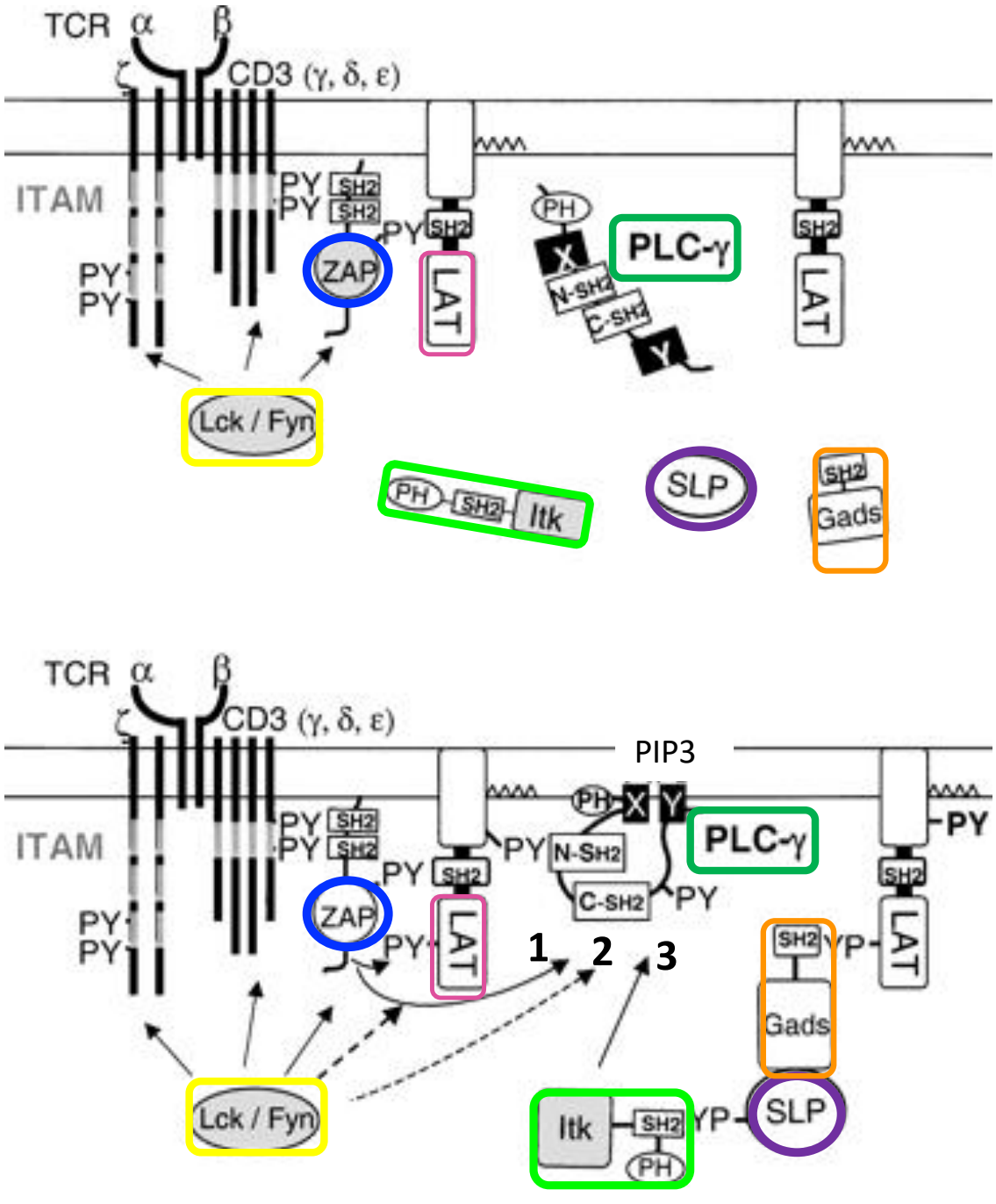
Enzyme activation is Ca^{++}-dependent



Phosphorylation of Y783 or 759 induces reorientation of the X-Y linker and activation of PLCγ



PLCγ1 in T lymphocyte activation

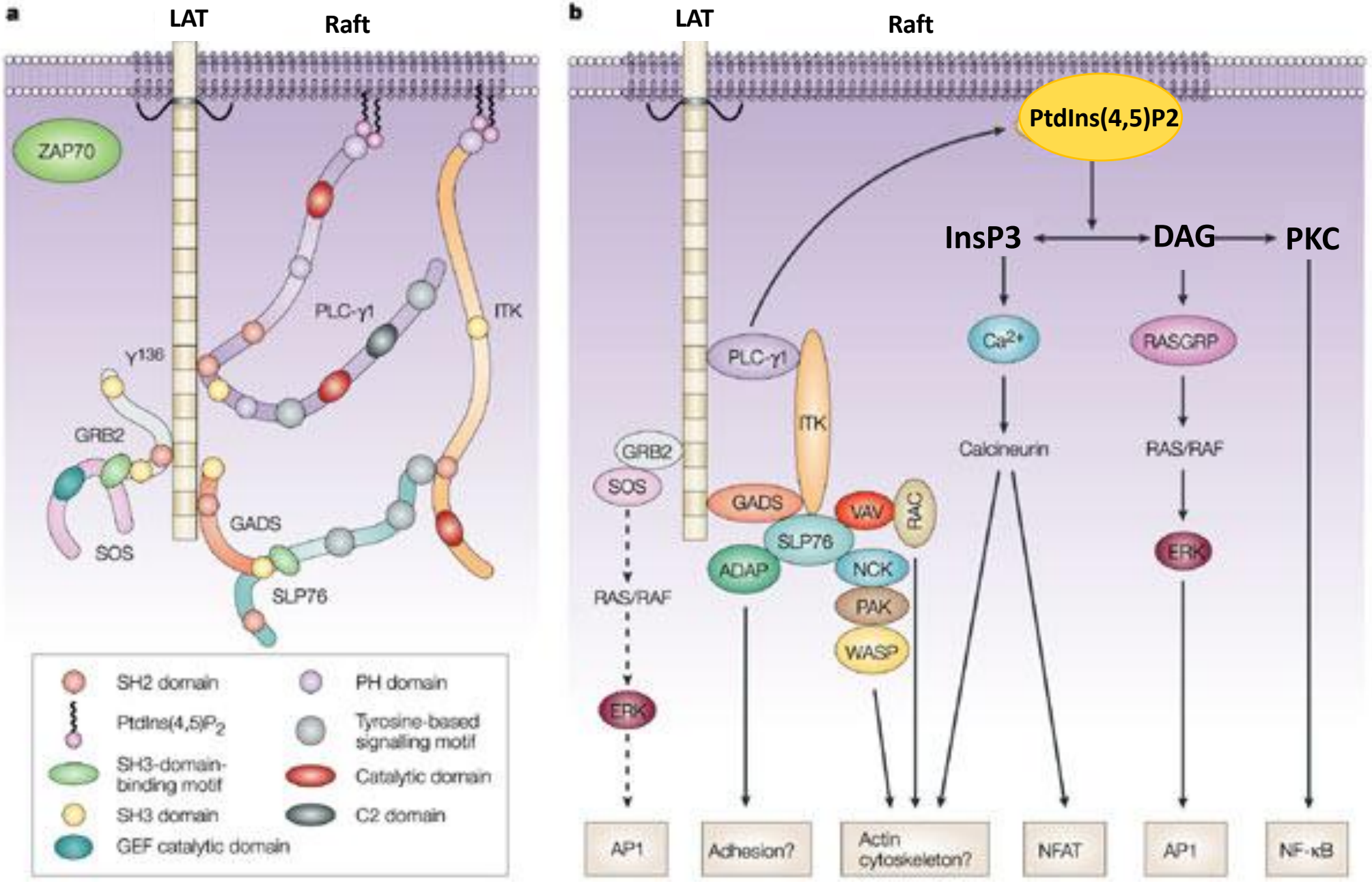


TCR-induced activation of PLCγ1

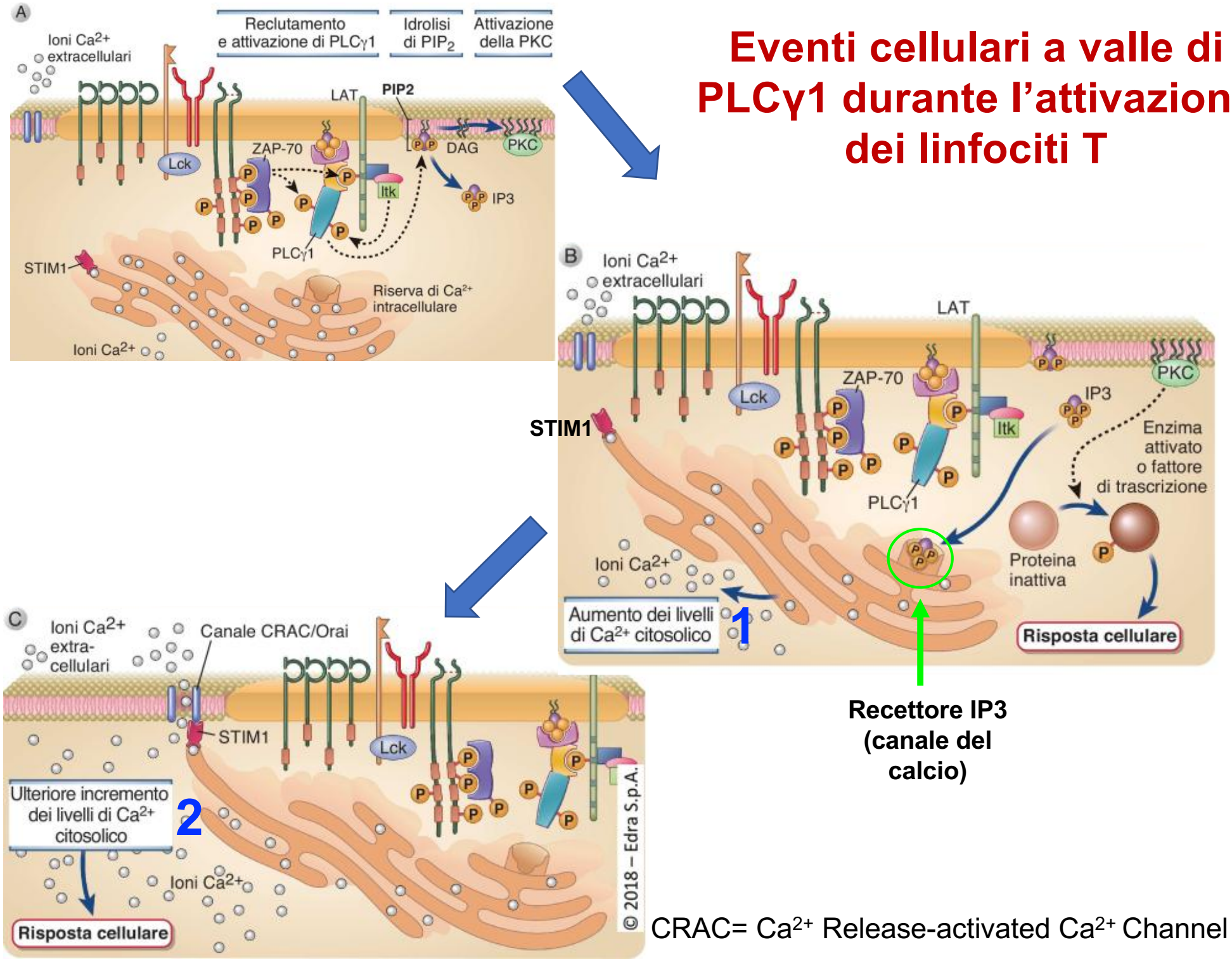
(Top) Ligation of the TCR triggers the activation of Lck and Fyn by unknown mechanisms. Either or both of these Src family PTKs then phosphorylates tyrosine residues within ITAM sequences located in TCR zeta and CD3 chains. Two phosphorylated tyrosine residues with this motif serve as binding sites or the tandem SH2 domains of ZAP-70. Lck or Fyn then phosphorylates the bound ZAP-70, resulting in its activation.

(Bottom) Together with Lck and Fyn, activated **ZAP-70** phosphorylates various downstream substrates, including membrane-bound **LAT** and SLP-76. The interaction of the N-SH2 domain of **PLCγ1** with a phosphorylated tyrosine residue of **LAT** serves to position the unphosphorylated enzyme close to activated ZAP-70 and Lck or Fyn, resulting in the phosphorylation and activation of PLCγ1 and in its localization in the vicinity of its substrate. Phosphorylated LAT also associates with **Gads**, which might in turn associate with Itk-bound **SLP-76**; the close proximity of **Itk** and PLCγ may result in the phosphorylation by Itk of PLCγ1. Two LAT molecules are shown to avoid overcrowding; this does not imply that PLCγ1 and Gads necessarily associate with separate LAT molecules. The EF-hand, SH3, and C2 domains of PLCγ1 are not shown.

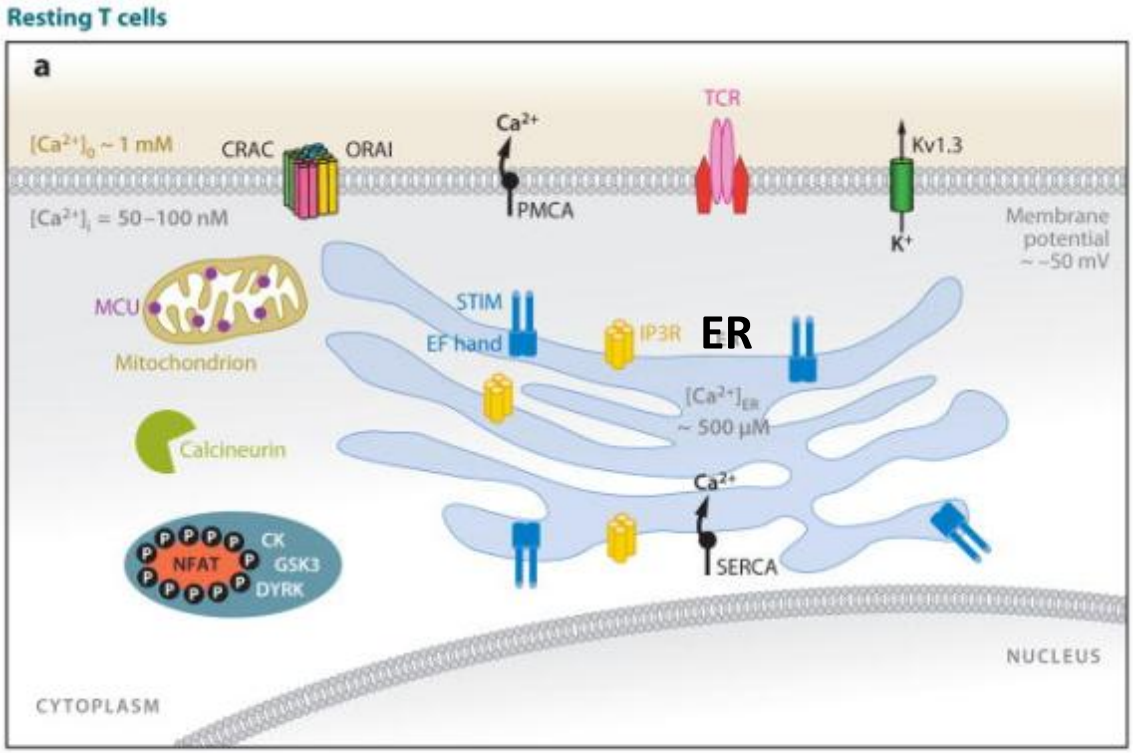
Reclutamento ed attivazione della PLCγ1

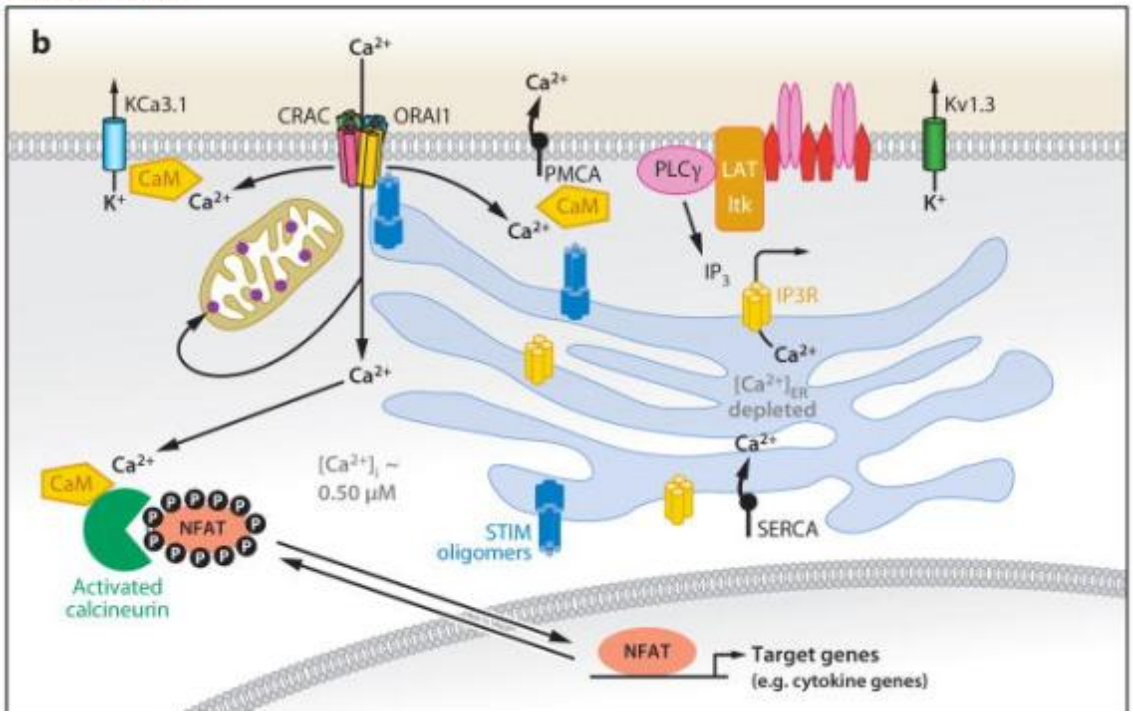


Eventi cellulari a valle di PLCγ1 durante l'attivazione dei linfociti T



CRAC= Ca^{2+} Release-activated Ca^{2+} Channel

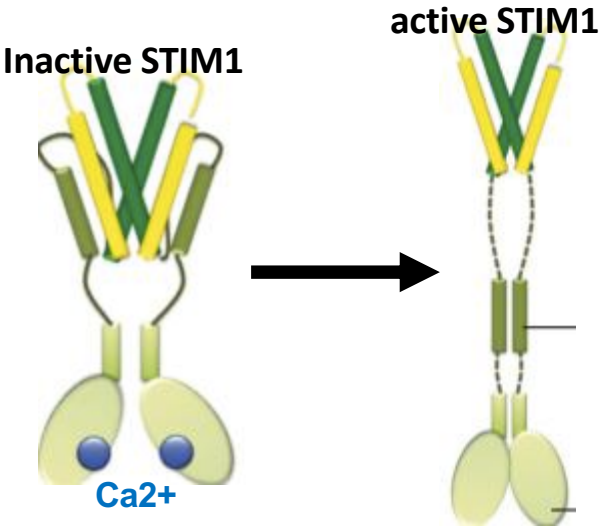




Ca^{2+} influx

1. Basal conditions STIM1 (stromal interaction molecule 1) is dimeric.

2. Following depletion of Ca2+ from **ER**, STIM1 goes through a conformational change and then oligomerizes.

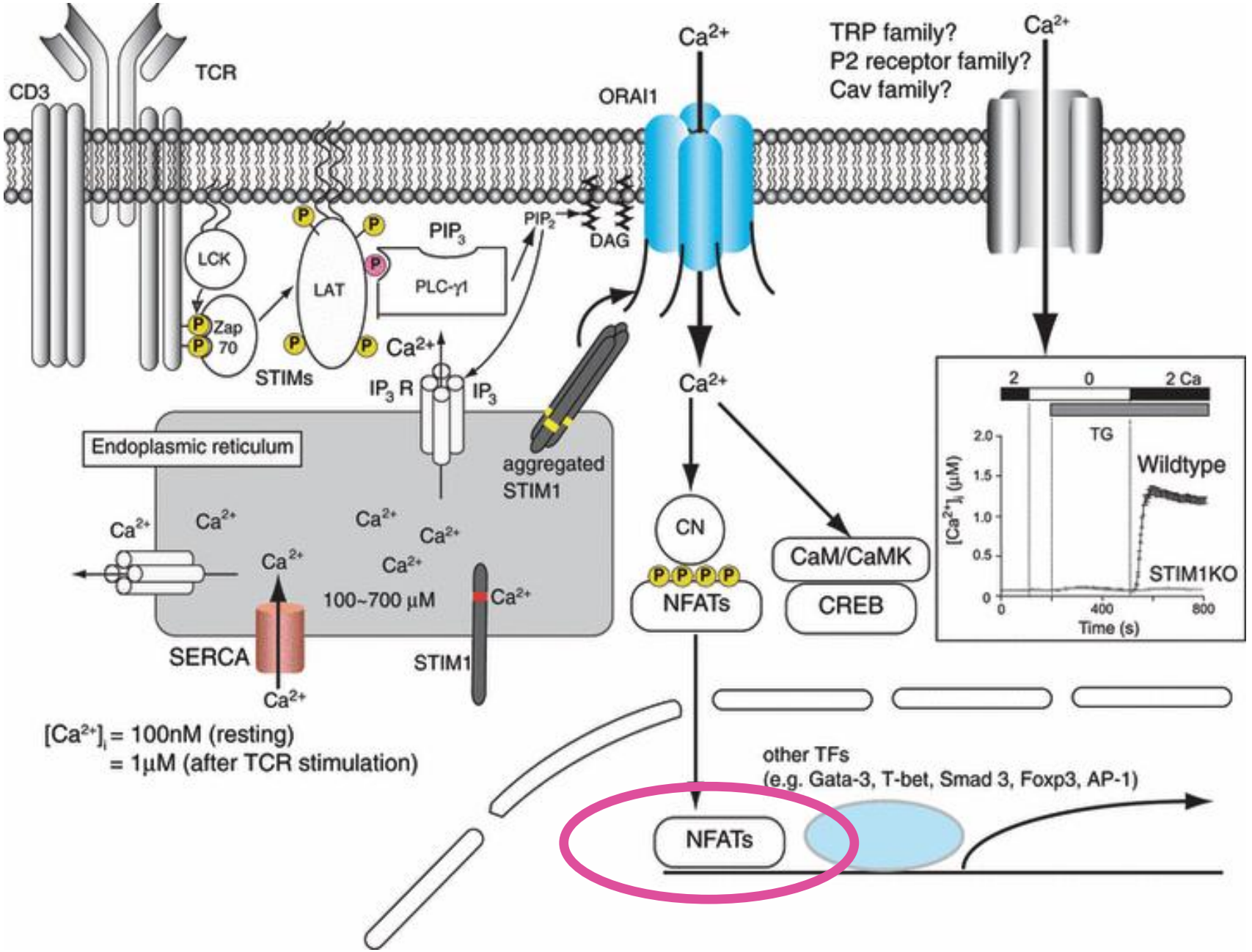


3. Oligomerization of STIM1 in the ER membrane is followed by relocalizion to ER-plasma membrane junctions.

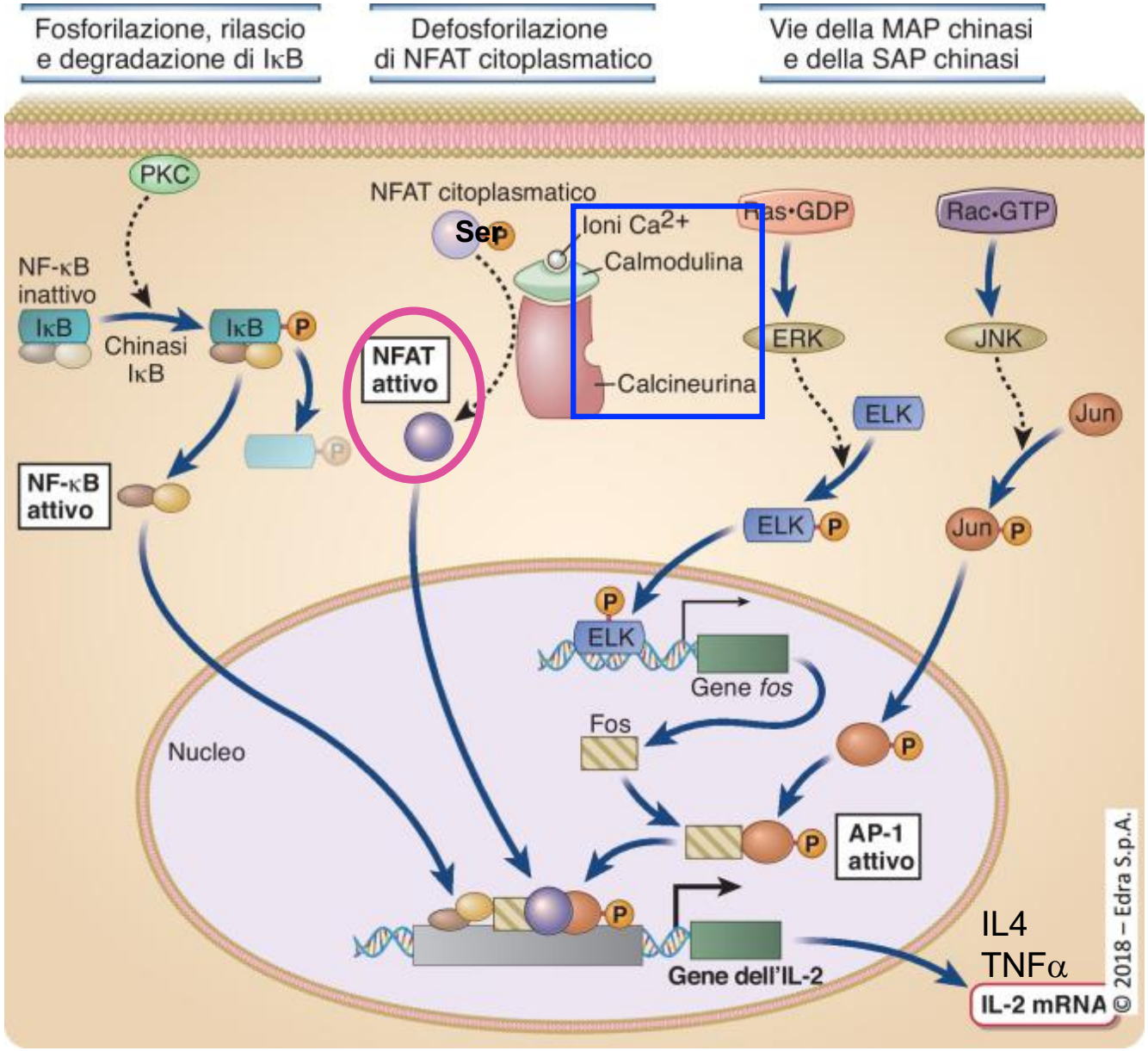
4. STIM1 oligomers then recruit **ORAI1** (structural component of the CRAC channel) by binding a C-terminal region of ORAI1.

5. STIM1 oligomers open **CRAC/ORAI1 channels** > high **Ca2+ influx**

IP3, Ca^{2+} ed attivazione di NF-AT



Attivazione dei fattori di trascrizione nei linfociti T: esempio di NFAT



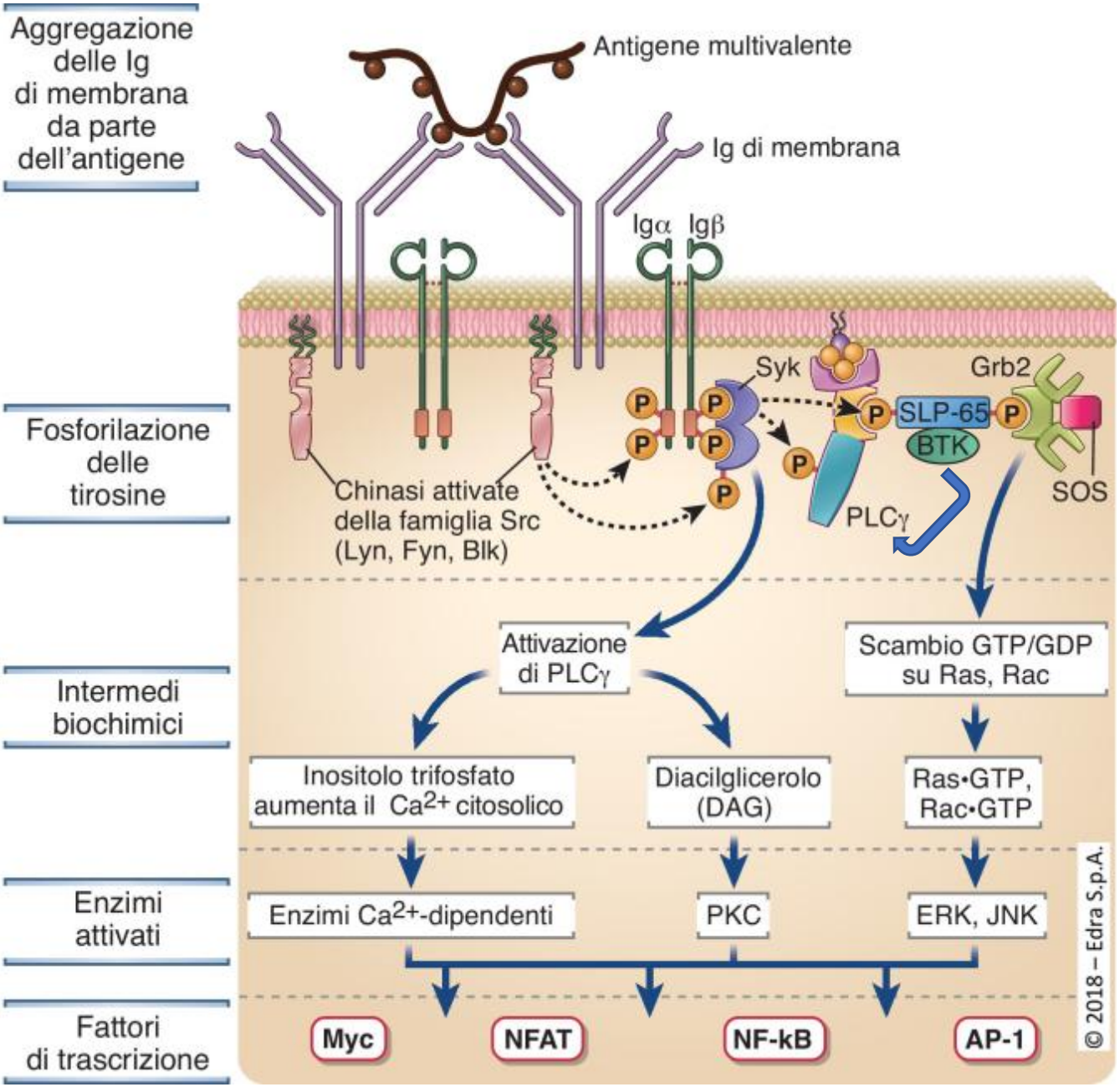
NFAT è presente nel citosol in forma inattiva fosforilata in Ser. E'attivato dalla **calcineurina** (fosfatasi Ca^{++}/calmodulina-dipendente) che defosforila NFAT permettendone la traslocazione nucleare.

La **ciclosporina** (farmaco immunosoppressivo) interagendo con la ciclofilina A forma un complesso che inibisce la calcineurina impedendo la traslocazione nucleare di NFAT.

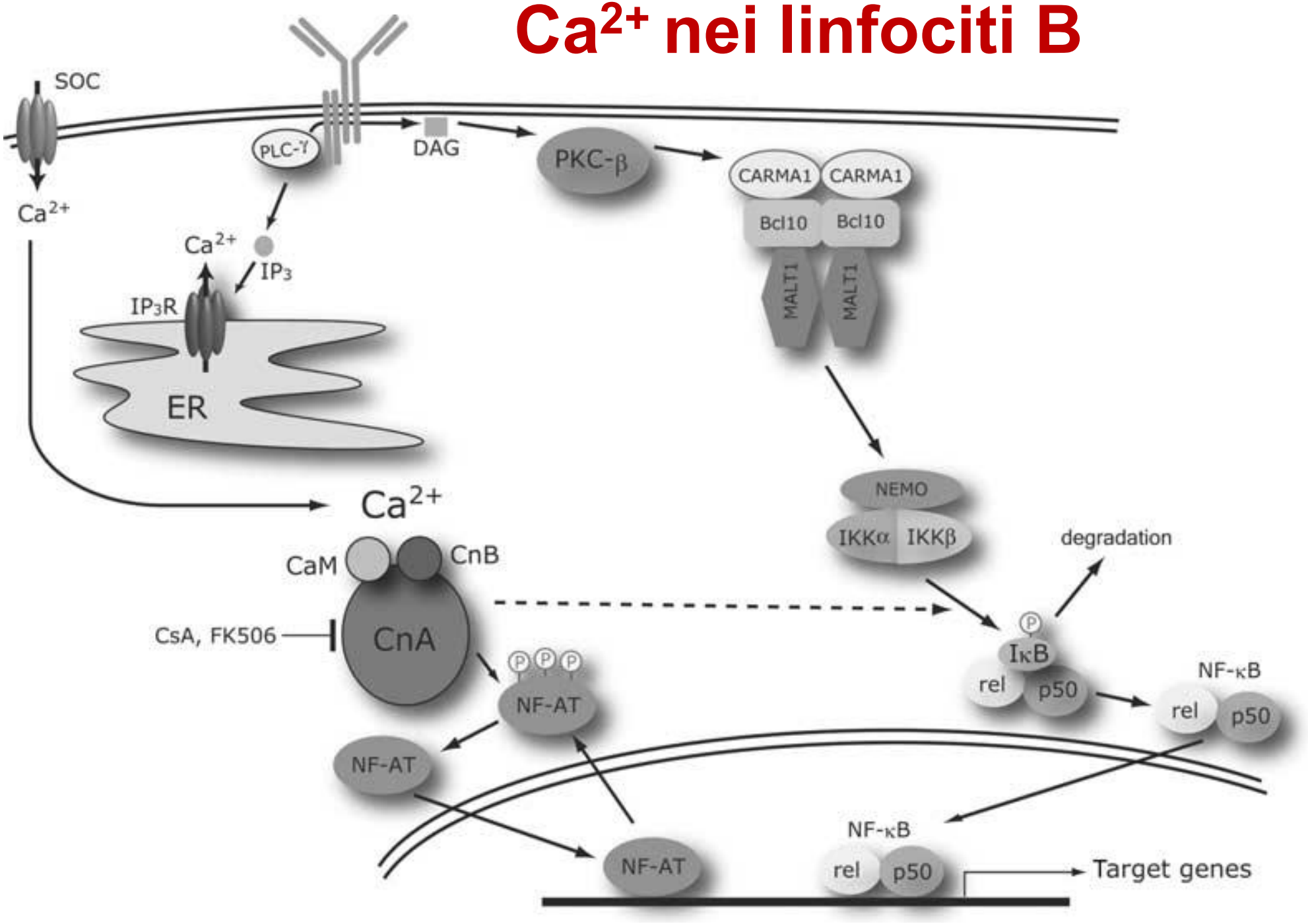
In modo analogo funziona il tacrolimus (**FK506**) che lega la FK506-binding protein.

L'effetto di questi farmaci è l'inibizione della trascrizione dei geni delle citochine nei linfociti T.

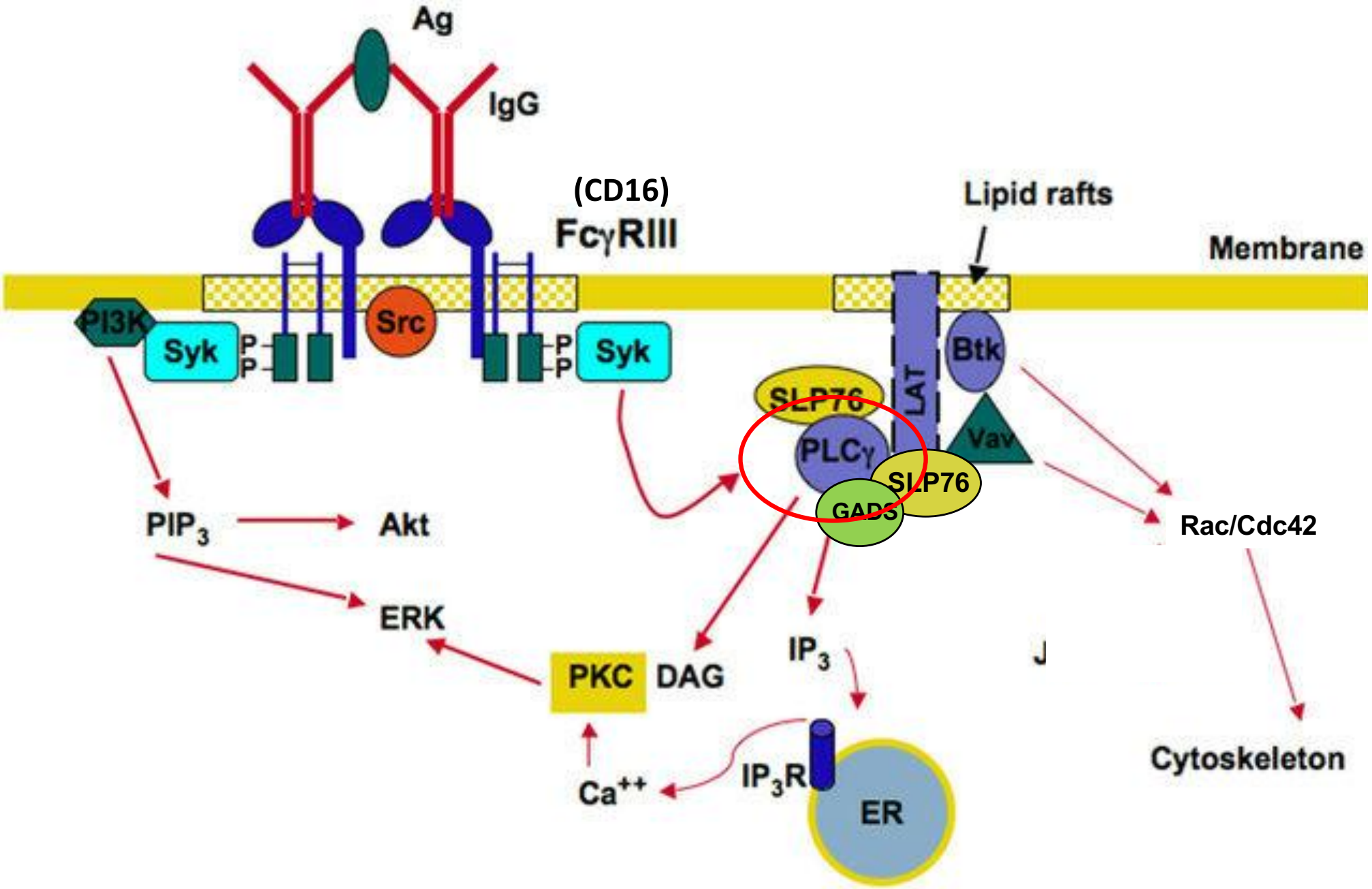
Attivazione della PLCγ2 nei linfociti B in seguito alla trasduzione del segnale via BCR



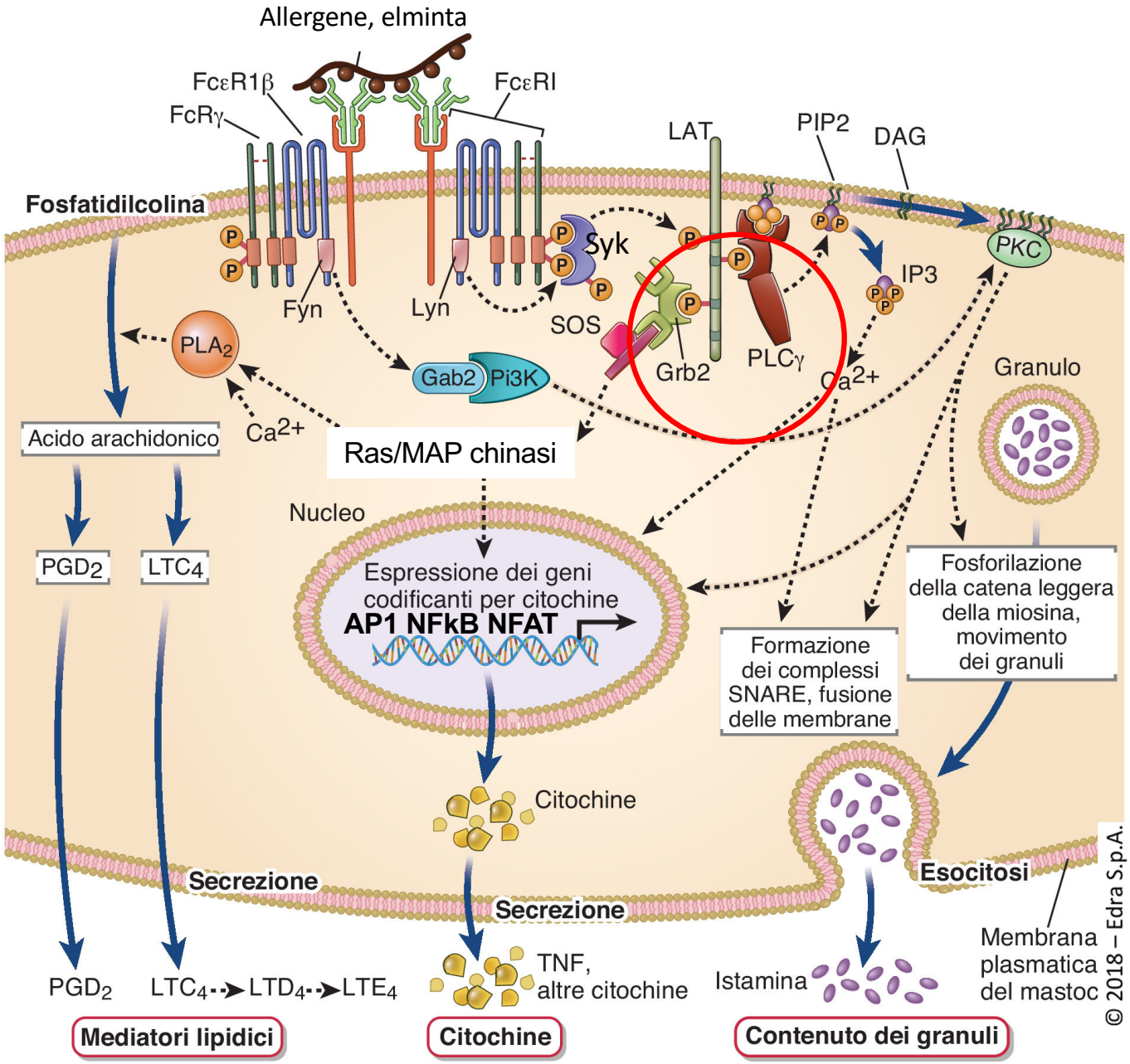
Ca^{2+} nei linfociti B



FcγRIII (CD16) e PLCγ nelle cellule NK



PLCγ e FcεRI nei mastociti



Mediatori contenuti nei granuli:

- **Istamina, eparina** e proteoglicani
- Proteasi neutre (triptasi, chimasi); idrolasi acide; catepsina G; carbossi-peptidasi

Mediatori *de novo*:

- Leucotrieni, PGD_2
- **Citochine:** TNF-α IL-3, IL-4, IL-5, IL-6
- Fattori chemiotattici per eosinofili e neutrofili