

# Phosphoinositide Signaling

**Pietro De Camilli**

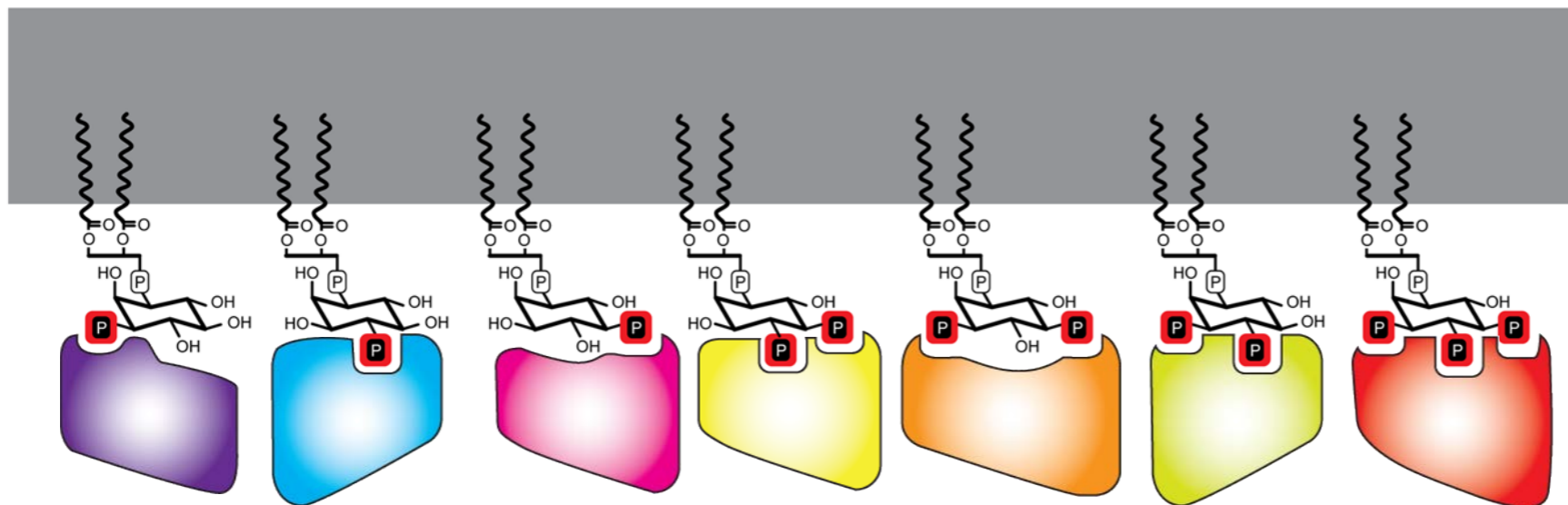
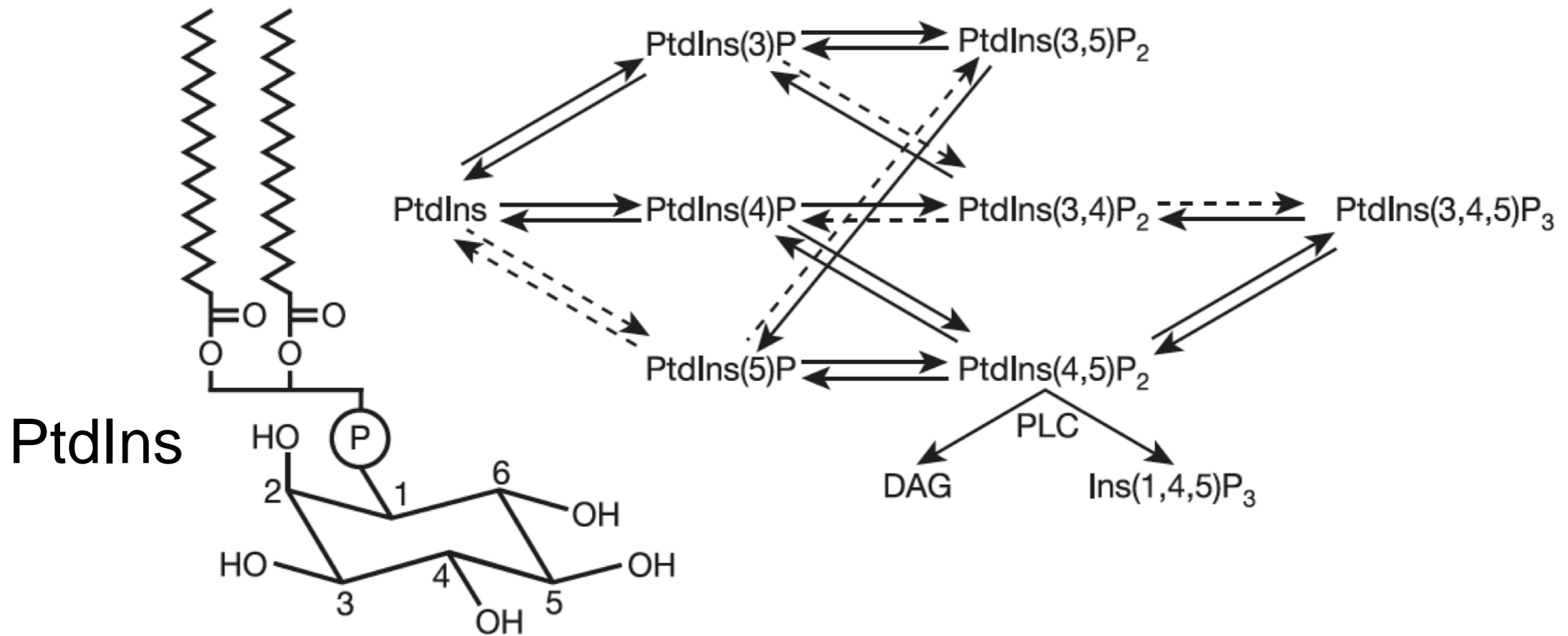
Department of Cell Biology

Howard Hughes Medical Institute

Program in Cellular Neuroscience, Neurodegeneration and Repair

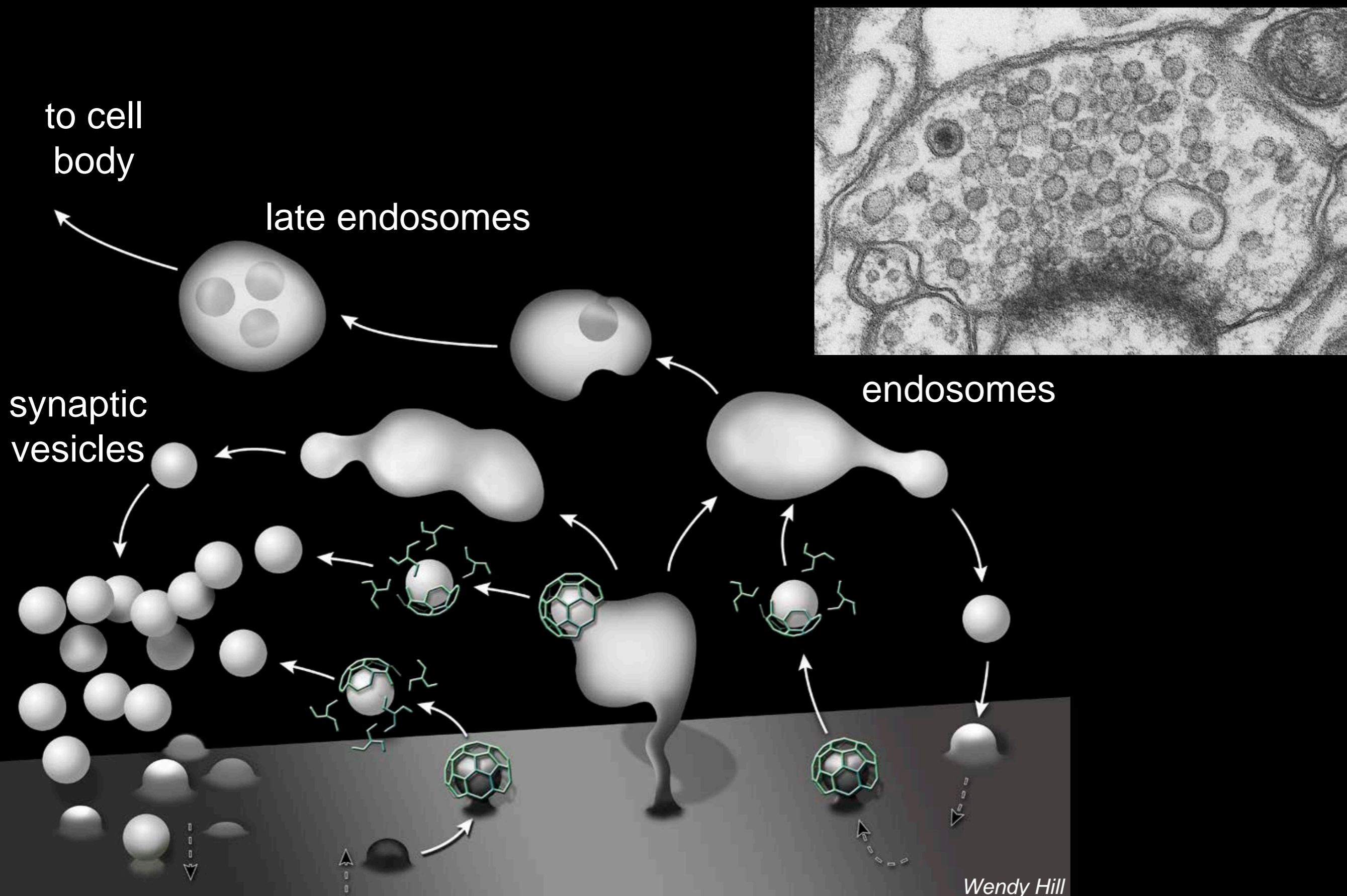
Yale University School of Medicine

# Phosphoinositides

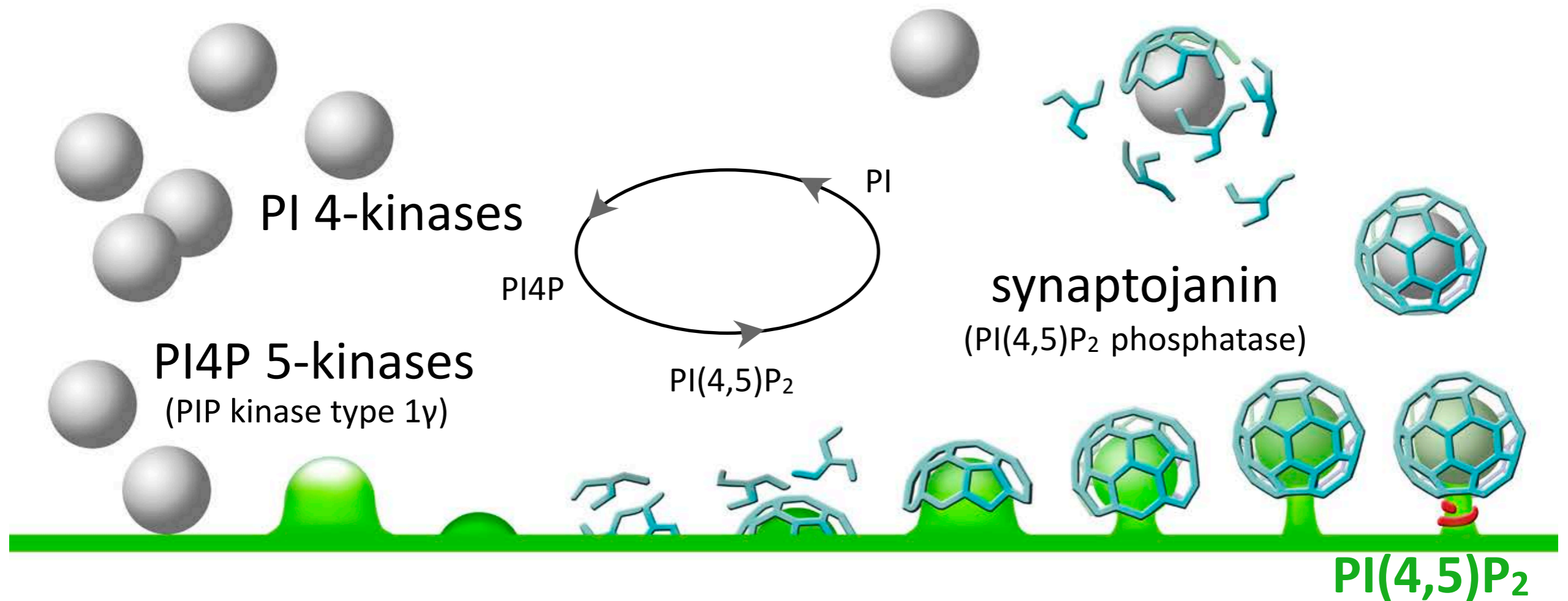


PH domains  
Fyve domains  
PX domains  
etc.

# Membrane traffic in nerve terminals

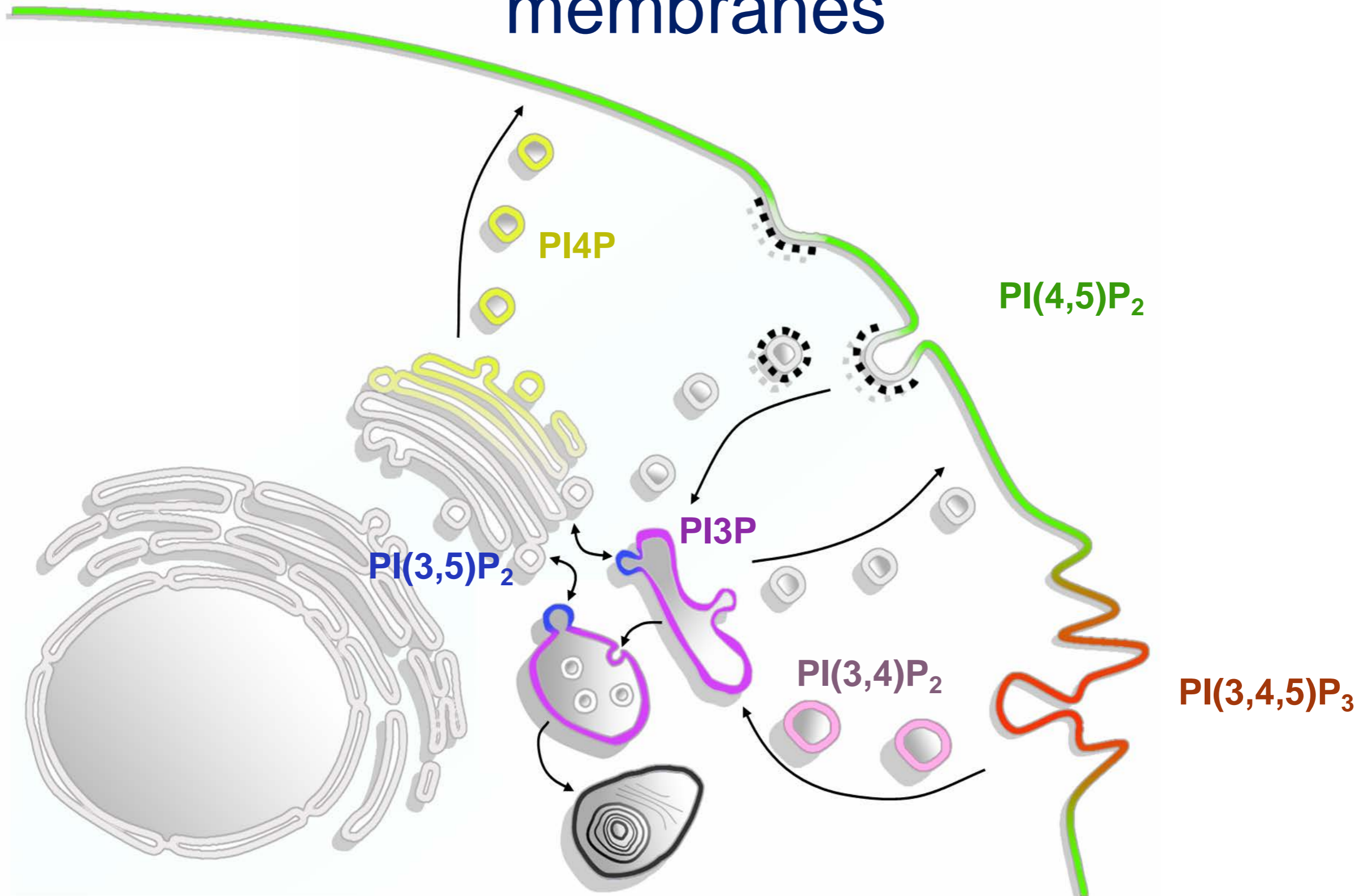


# A cycle of PI(4,5)P<sub>2</sub> synthesis and hydrolysis nested within the synaptic vesicle cycle

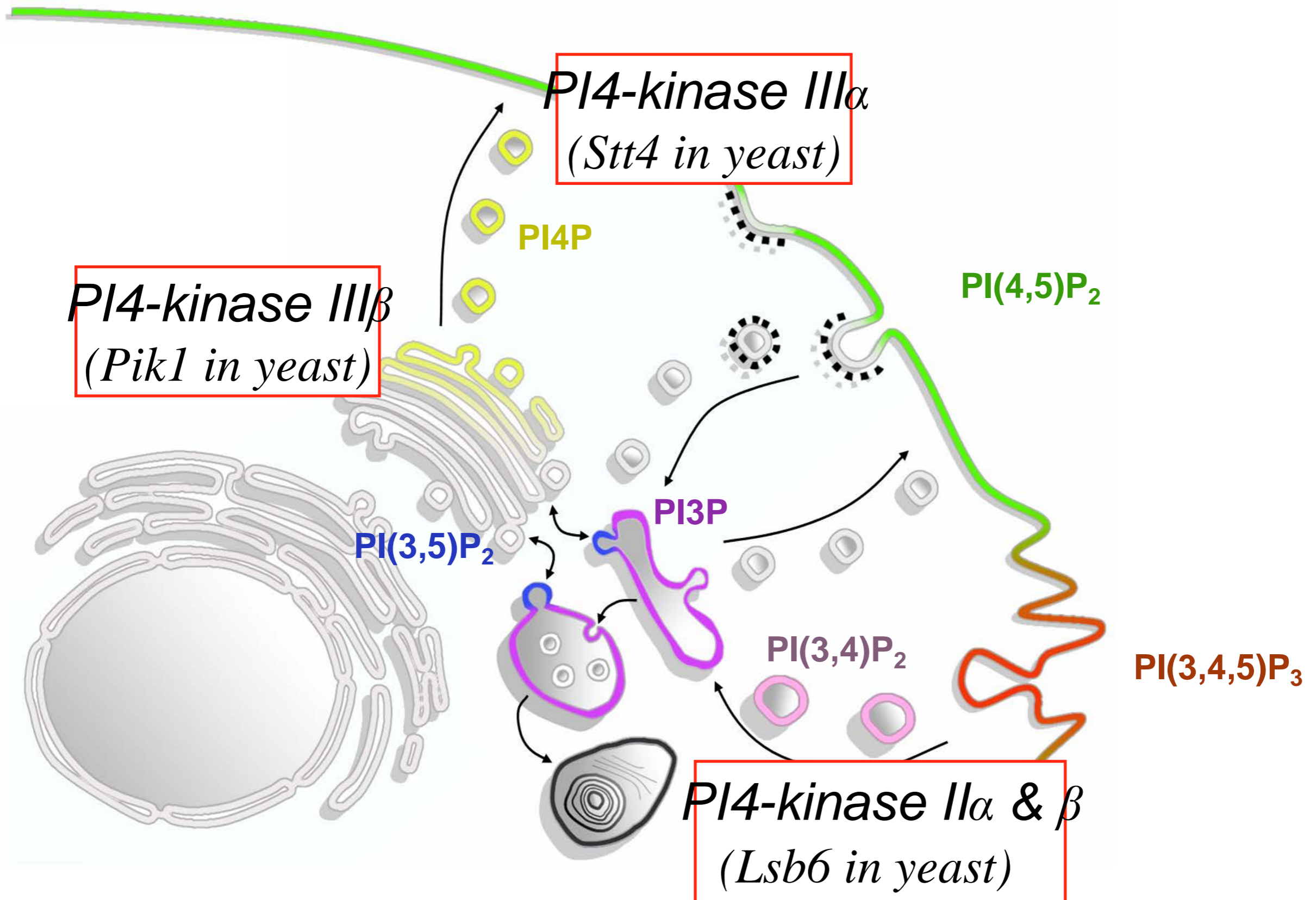




# A phosphoinositide code for cellular membranes

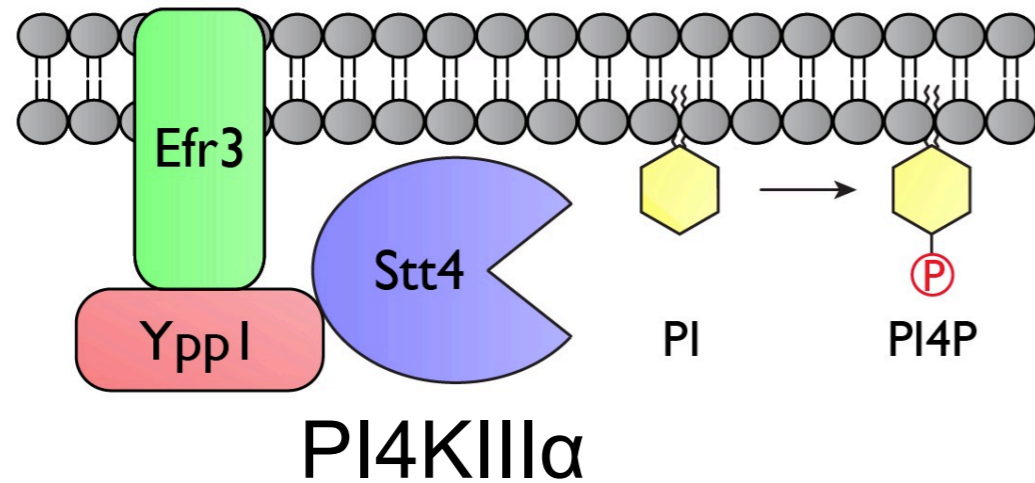


# Four PI 4-kinases in mammalian cells

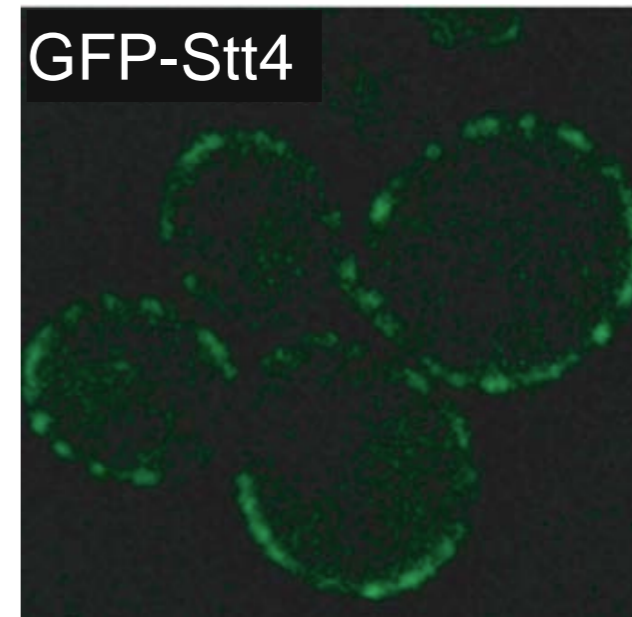




# Stt4 is targeted to the plasma membrane in a complex with Efr3 and Ypp1

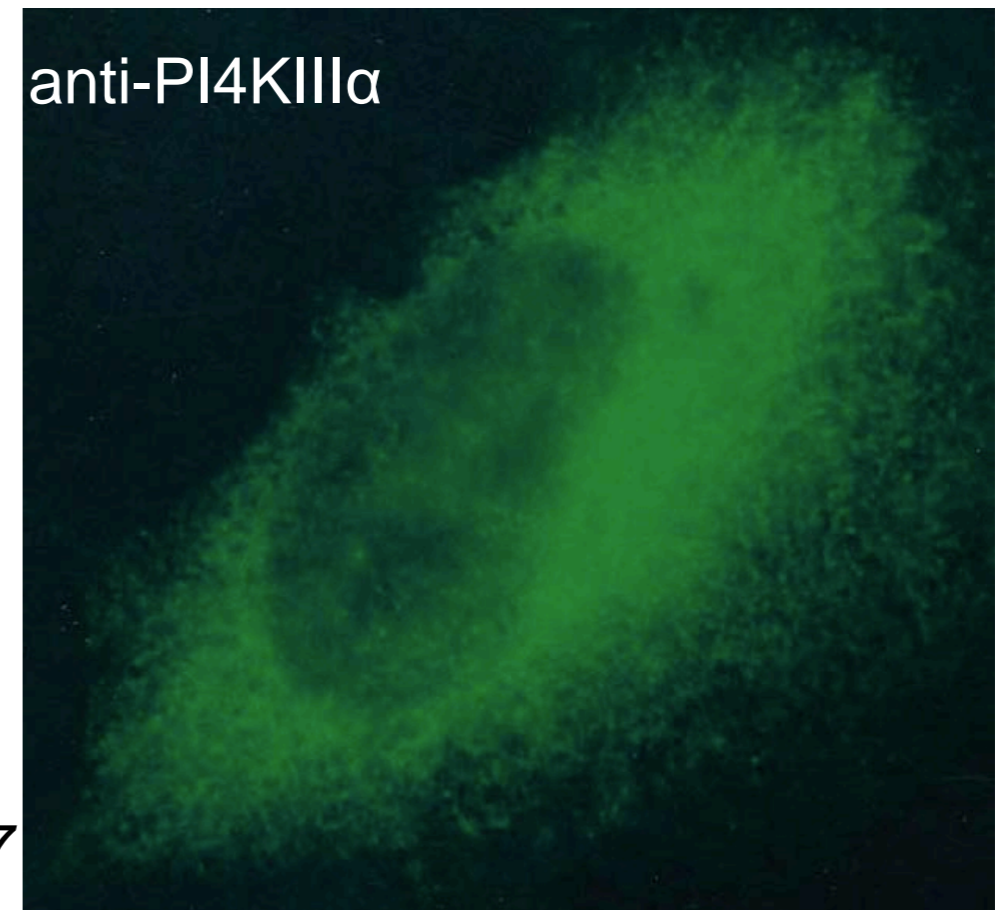


**Yeast**



Baird *et al.* (Emr lab), *J Cell Biol* 2008

**Mammalian cell**

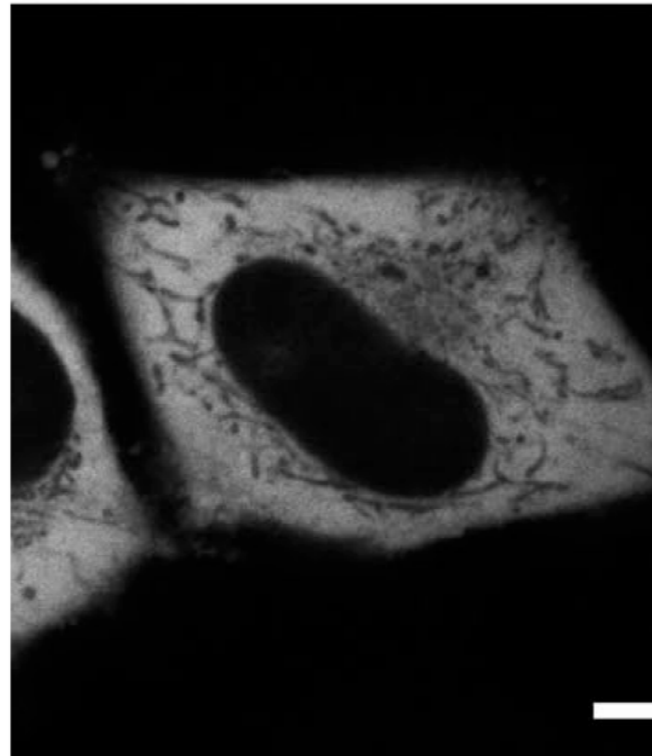


## Anti-PI4KIII $\alpha$ immunoprecipitation

Protein name	EFR3B	TTC7B
Accession number	NP_001075952	NP_001028385
Predicted molecular mass	92 kD	94 kD
No. unique peptides	5	12
Sequence coverage	9.3%	20.3%

Wong, (Cantley lab) *J. Biol. Chem.* 1997

# GFP-PI4KIII $\alpha$ is cytosolic



Based on the sequence reported in:

Nakagawa, T. , K. Goto , and H. Kondo . 1996 . Cloning, expression, and localization of 230-kDa phosphatidylinositol 4-kinase . J. Biol. Chem. 271 : 12088 –12094



# PI4KA (which encodes PI4KIII $\alpha$ ) had an incorrectly annotated translation start site

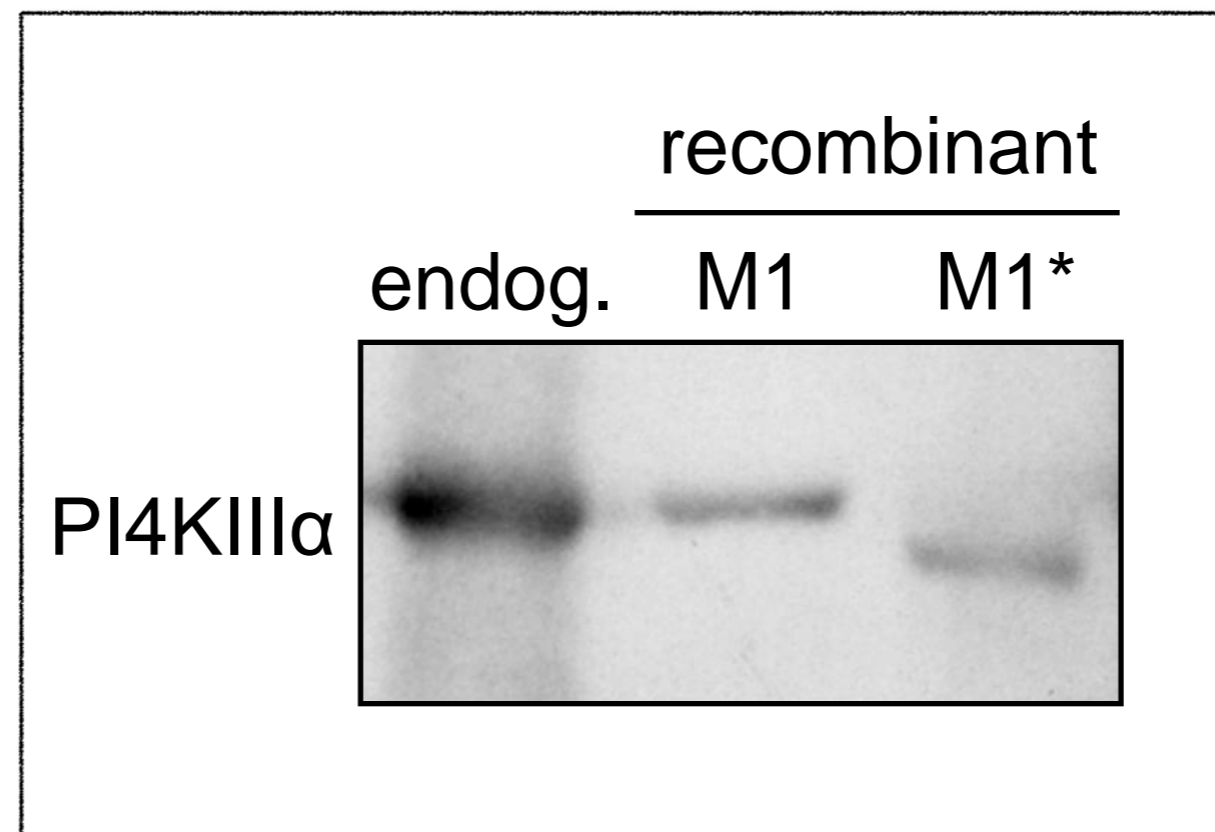
M1

M1\*

<i>Homo</i>	MAAAPARGGGGGGGGGGGCSG---SGSSASRGFYFNTVLSLARSLAVQRPASLEKVQKLLCMCPVDFHGI FQLDERRRDAVIALGI
<i>Pan</i>	MAAAPARGGGGG---GGCSG---SGSSASRGFYFNTVLSLARSLAVQRPASLEKVQKLLCMCPVDFHGI FQLDERRRDAVIALGI
<i>Canis</i>	MAAAAARGGGGGSSGGGG---SSSGSSSRGFYFNTVPSLARSLAVQRPASLEKVQKLLCMCPVDFHGI FQLDERRRDAVIALGI
<i>Rattus</i>	MAAAGARGTGGSG-----SSSGSSTSRGFYFNTVPSLARSLAVQRPASLEKVQKLLCMCPVDFHGI FQLDERRRDAVIALGI
<i>Mus</i>	MAAAGARGGGGGGGGGGGGGSGSSSGSSTSRGFYFNTVLSLARSLAVQRPASLEKVQKLLCMCPVDFHGI FQLDERRRDAVIALGI
<i>Danio</i>	-----MATRDFYFNTVLSLARSLAAHRPAPVEKVQKLLCMCPCD SRGVFVLDVRRRDAVIALAV

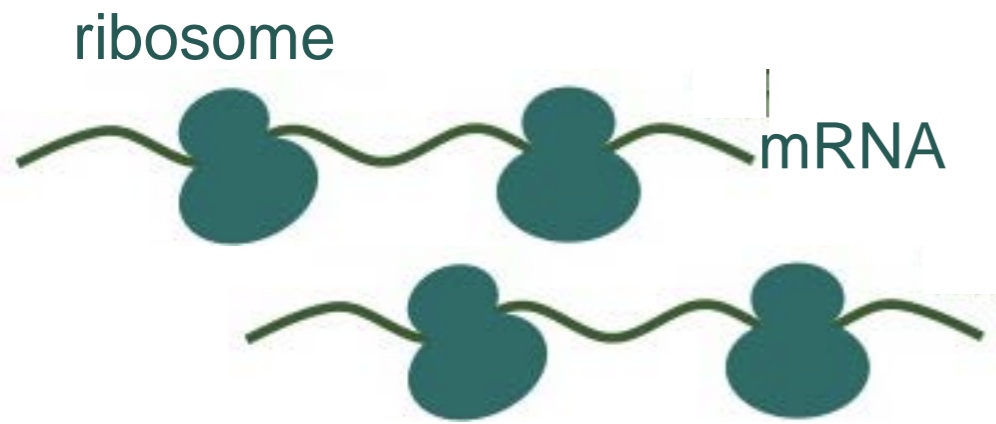
↑  
likely the correct start site

↑  
annotated start site



# Ribosome footprinting after harringtonine treatment enabled determination of PI4KIII $\alpha$ translational start sites

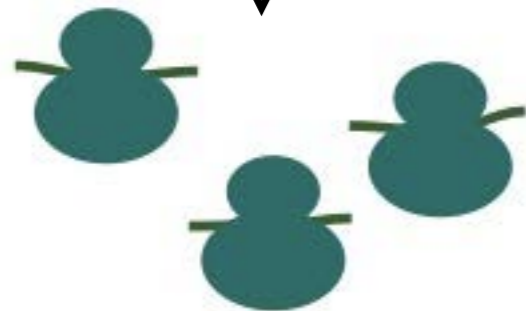
Method in: Ingolia et al. (Weissman lab), Cell 2011



Harringtonine



Nuclease digestion



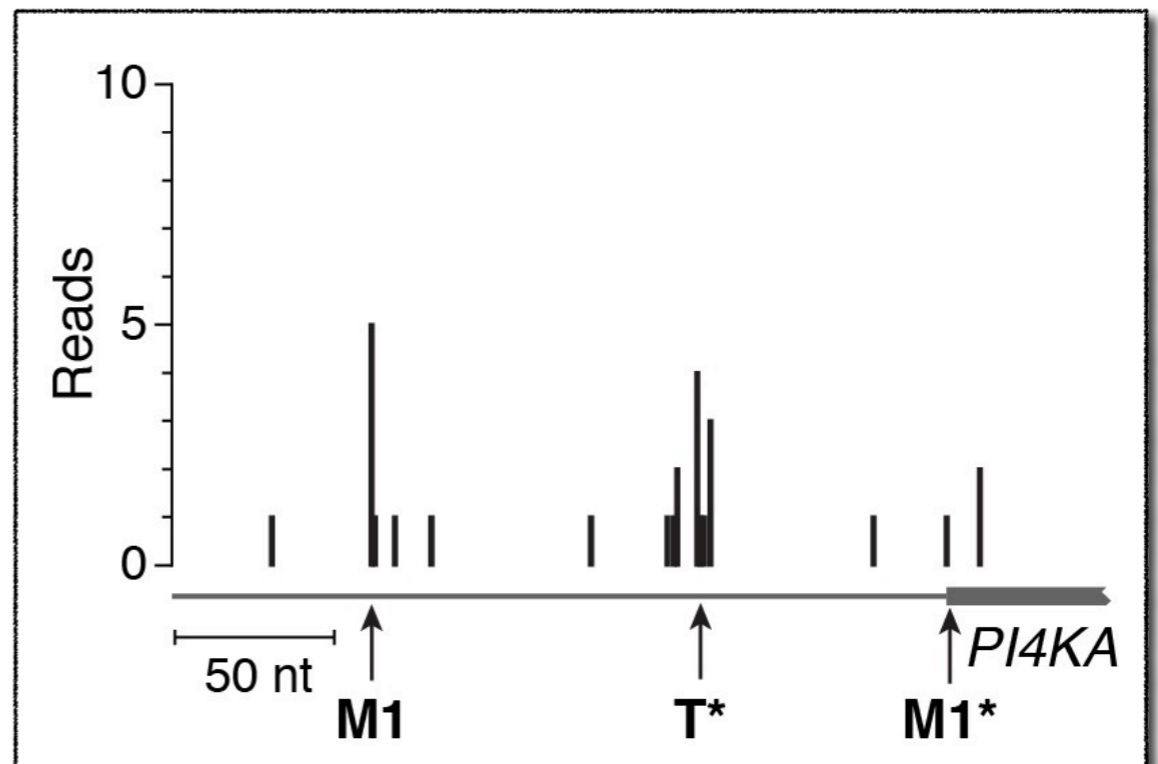
Deep sequencing library

<i>Homo</i>	MAAAPARGGGGGGGGGGCSG---	SGSSASRGFYFNTVLSLARSLAVQRPASLEKVQKLLCM
<i>Pan</i>	MAAAPARGGGGGGGGGGCSG---	SGSSASRGFYFNTVLSLARSLAVQRPASLEKVQKLLCM
<i>Canis</i>	MAAAAARGGGGGSSGGGGG---	SSSGSSASRGFYFNTVPSLARSLAVQRPASLEKVQKLLCM
<i>Rattus</i>	MAAAGARGTGGSG-----	SSSGSSTSRGFYFNTVPSLARSLAVQRPASLEKVQKLLCM
<i>Mus</i>	MAAAGARGGGGGGGGGGGGSGSSSGSSTSRGFYFNTVLSLARSLAVQRPASLEKVQKLLCM	
<i>Danio</i>	-----	MATRDFYFNTVLSLARSLAAHRPAPVEKVQKLLCM

M1

T\*

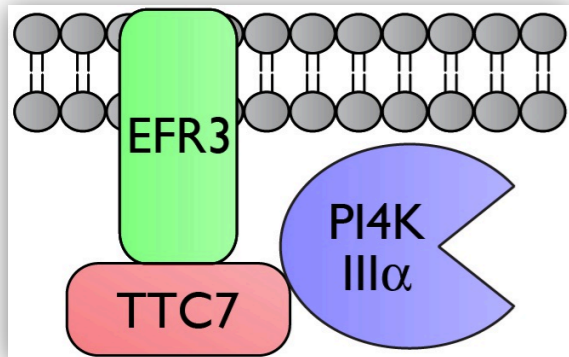
M1\*



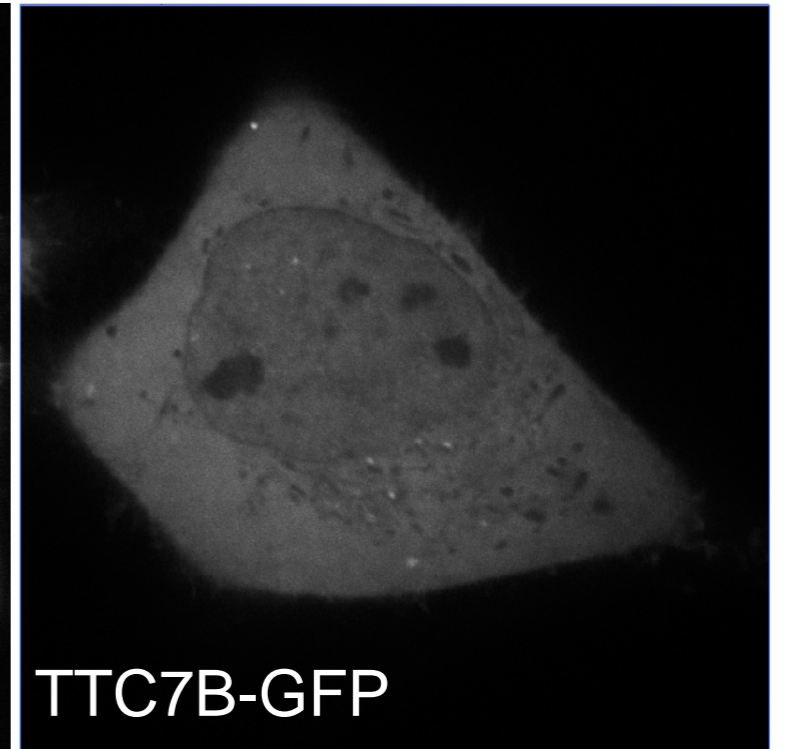
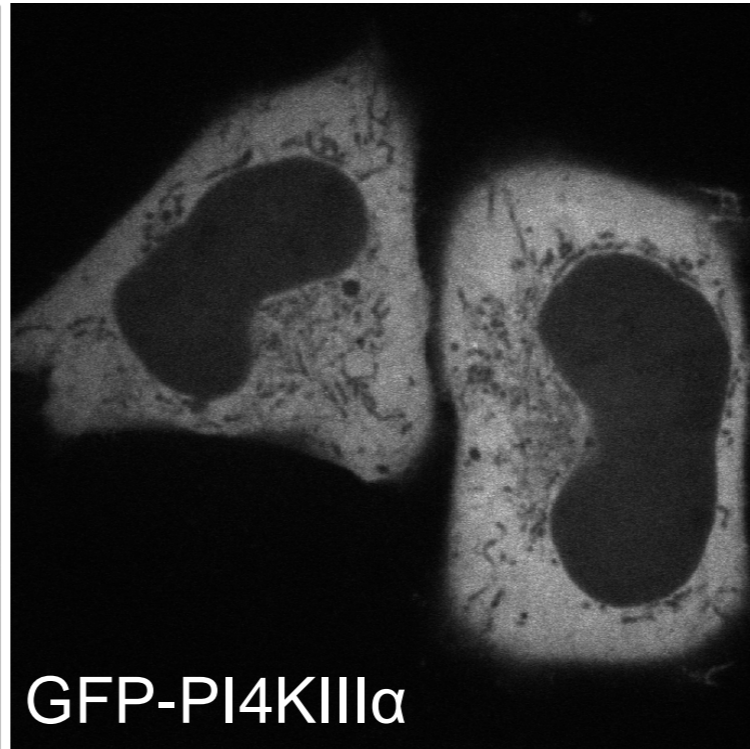
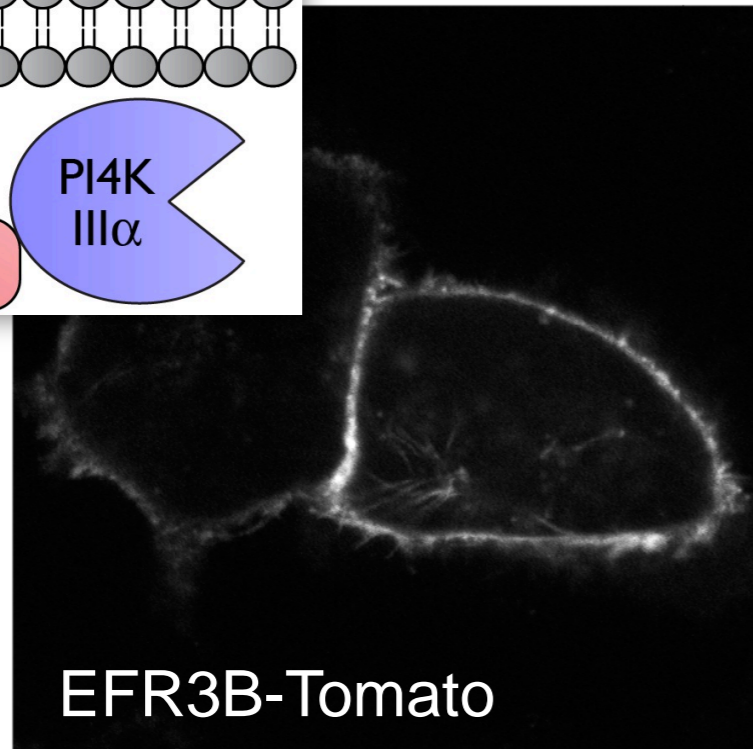
collaboration with N. Ingolia's lab (Carnegie Institute)



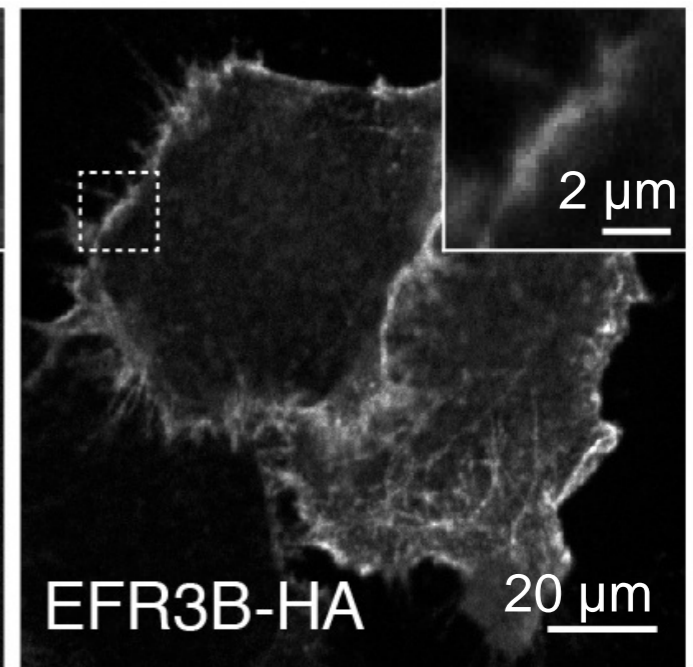
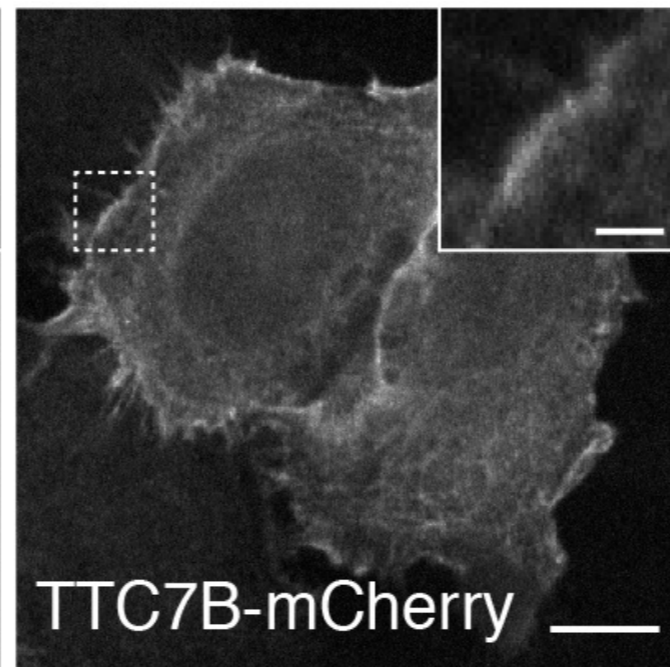
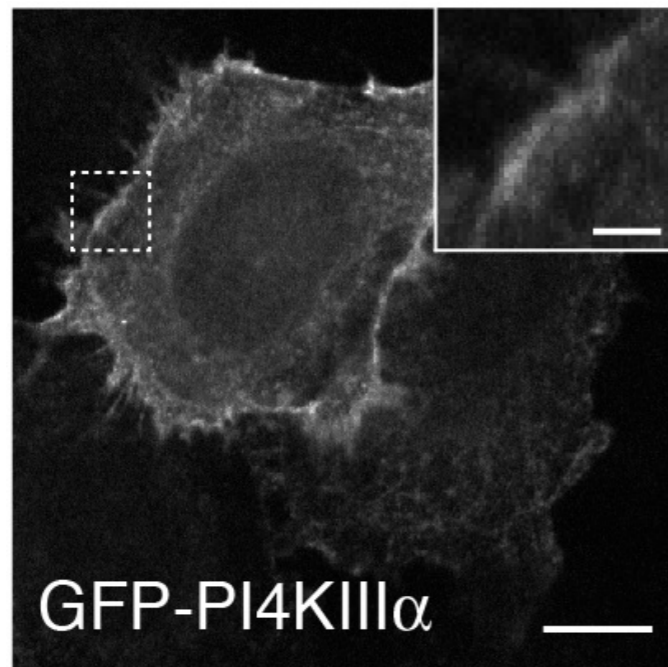
# A PI4KIII $\alpha$ /EFR3/TTC7 complex as in yeast



alone



together



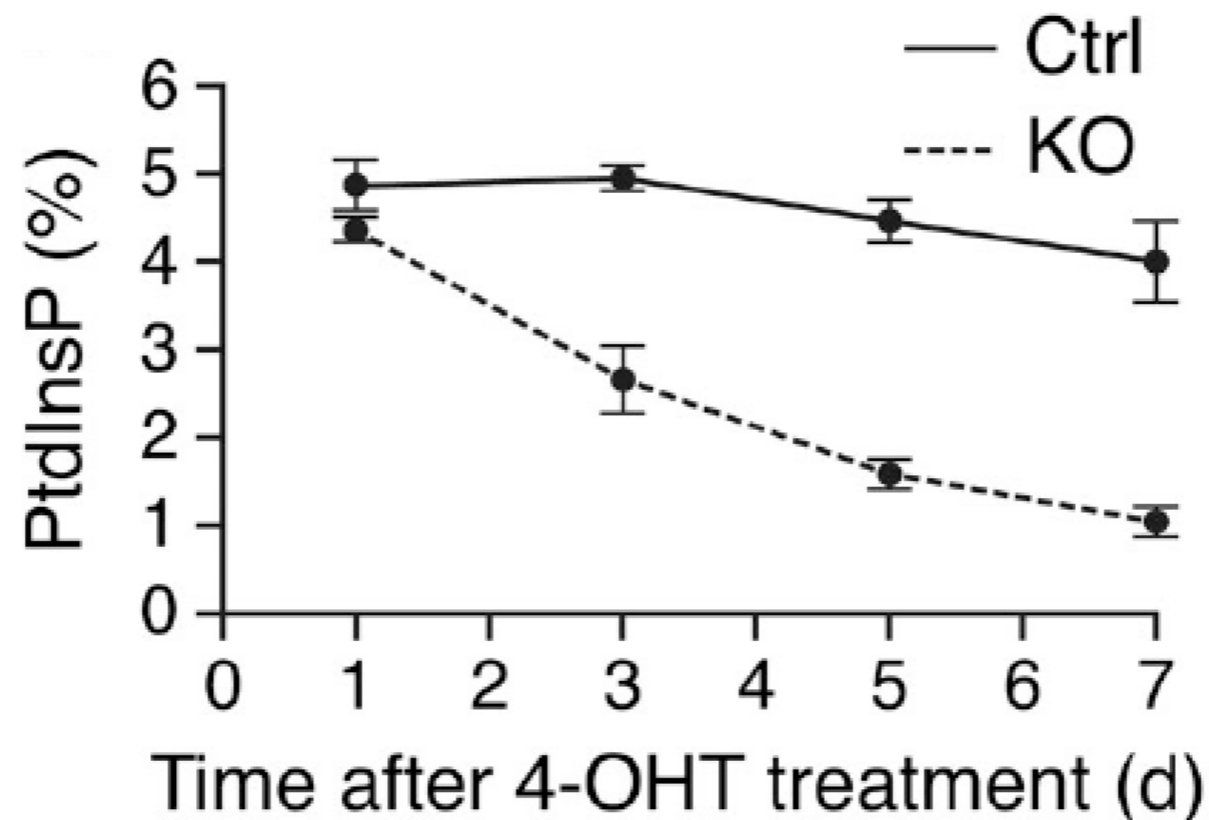
**What is the function of this complex ?**

# Conditional PI4KIII $\alpha$ KO mice

Germline gene disruption = embryonic lethality

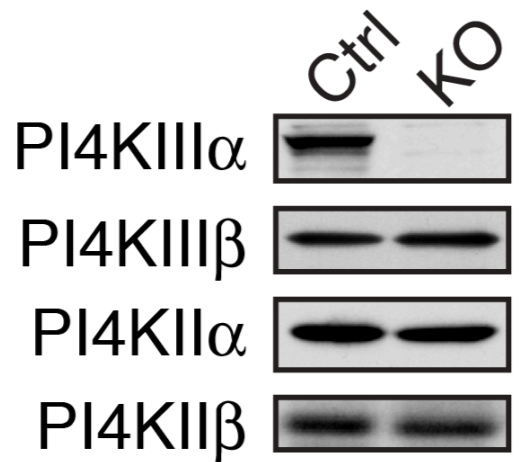
Conditional KO  $\rightarrow$  crossed to CreER expressing mouse  
then isolation of mouse embryonic fibroblasts (MEFs) + tamoxifen

conditional PI4KIII $\alpha$  MEFs

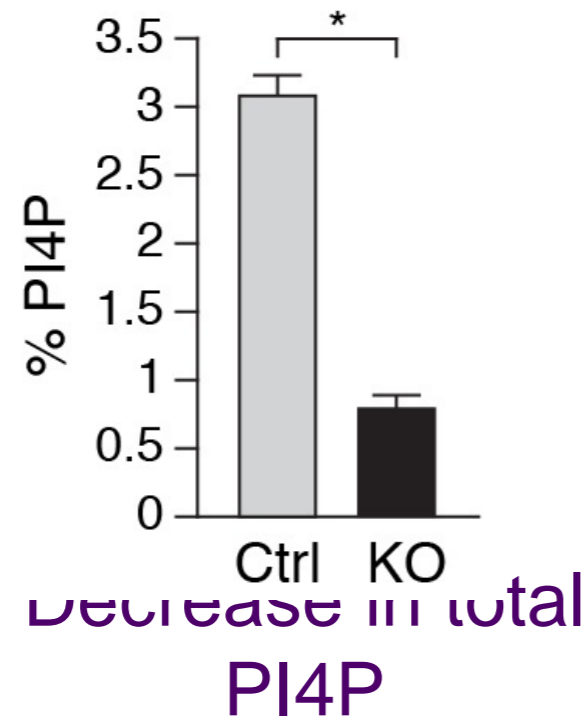




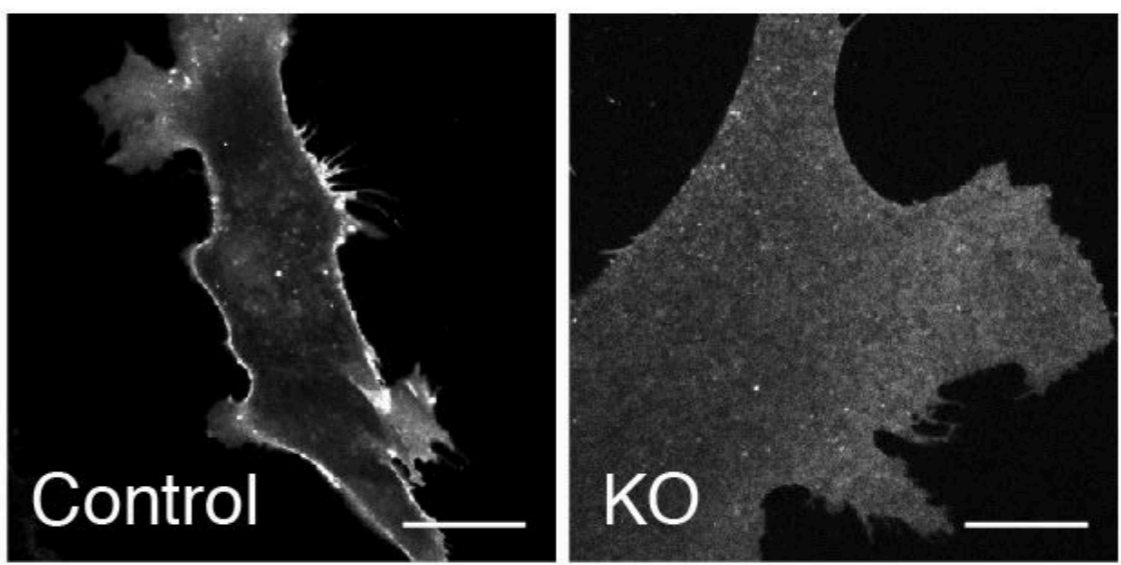
# PI4KIII $\alpha$ KO MEFs confirm central role of this kinase in plasma membrane PI4P synthesis



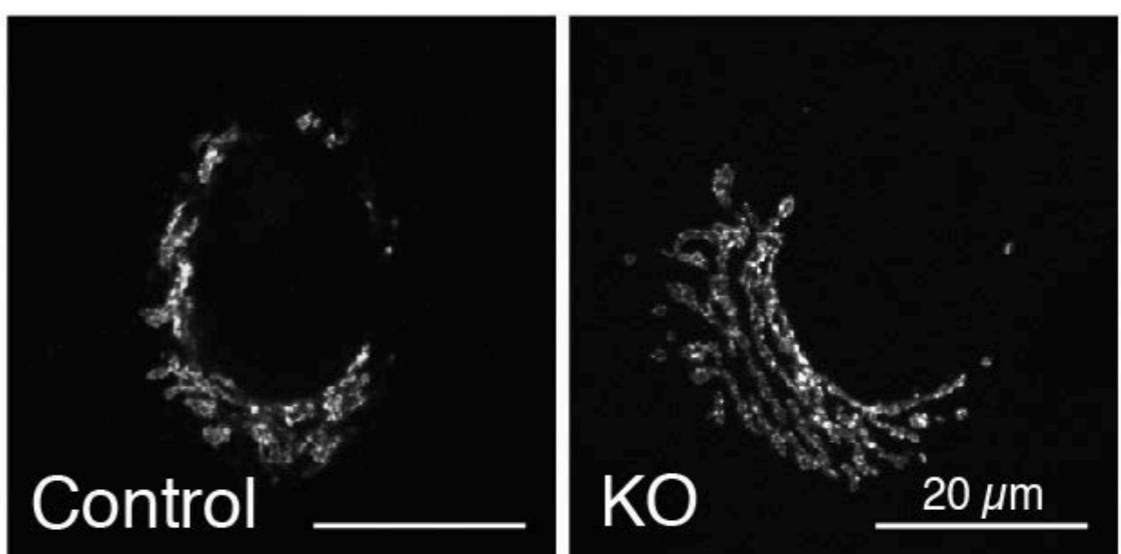
No change in levels of other PI 4-kinases



GFP-PH<sup>Osh2</sup>

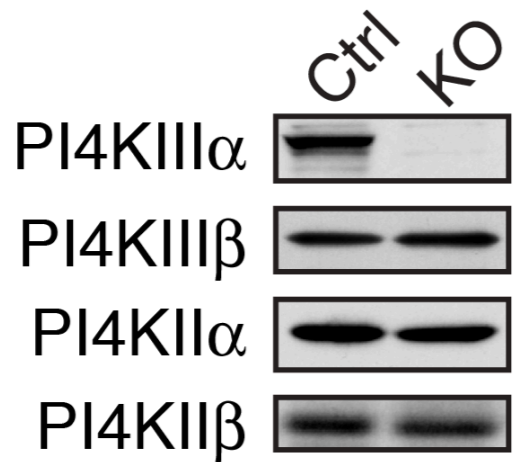


GFP-GOLPH3

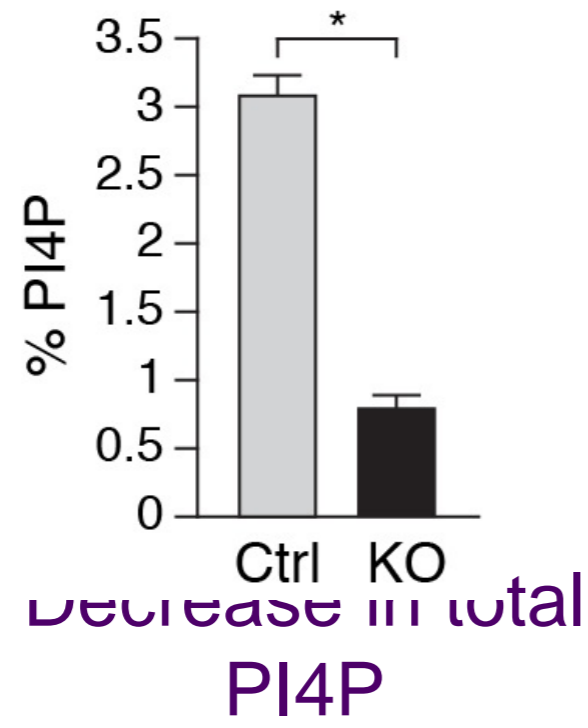


Decrease in PI4P at the plasma membrane but not at the Golgi apparatus

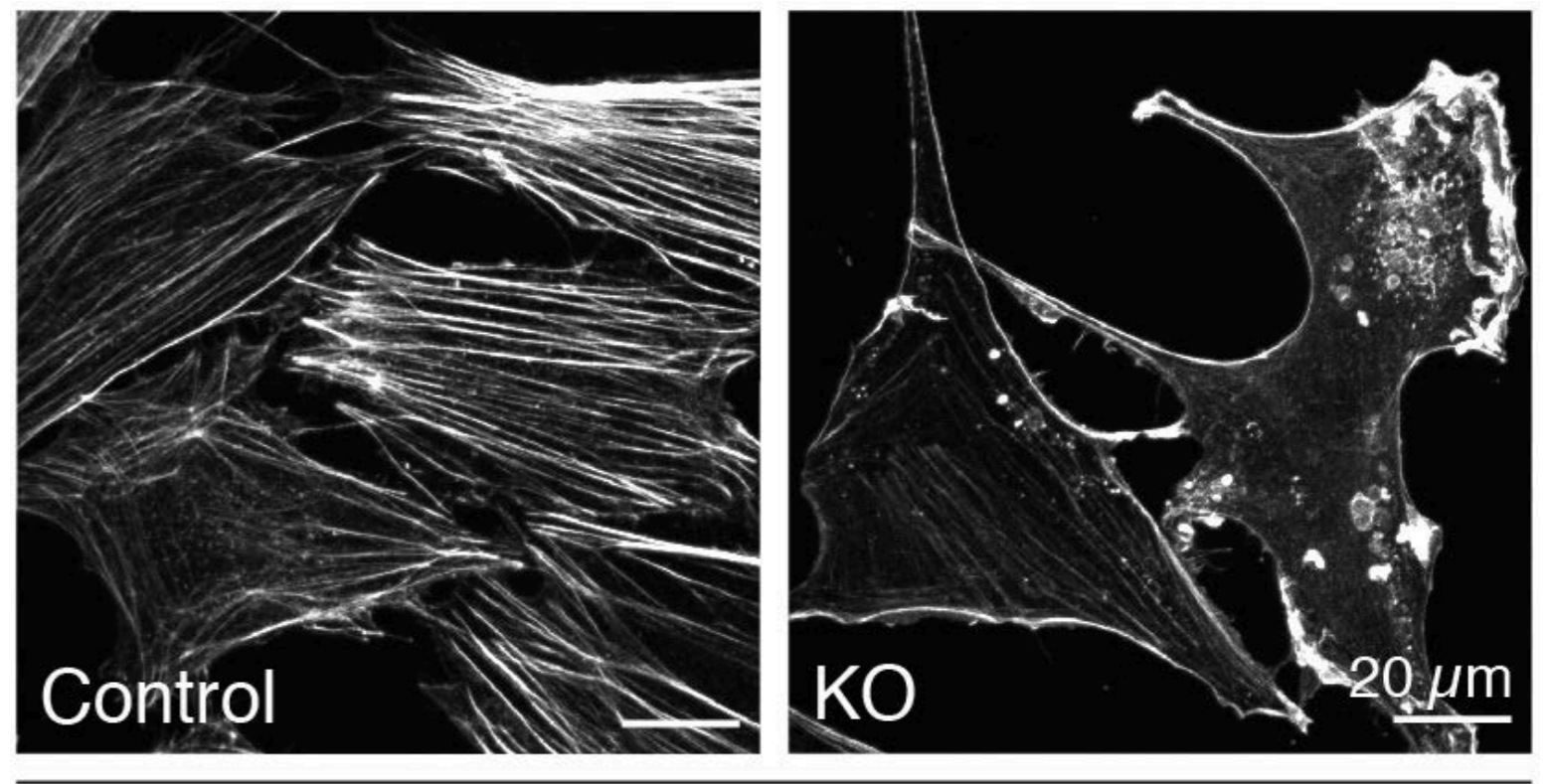
# PI4KIII $\alpha$ KO MEFs confirm central role of this kinase in plasma membrane PI4P synthesis



No change in levels of other PI 4-kinases

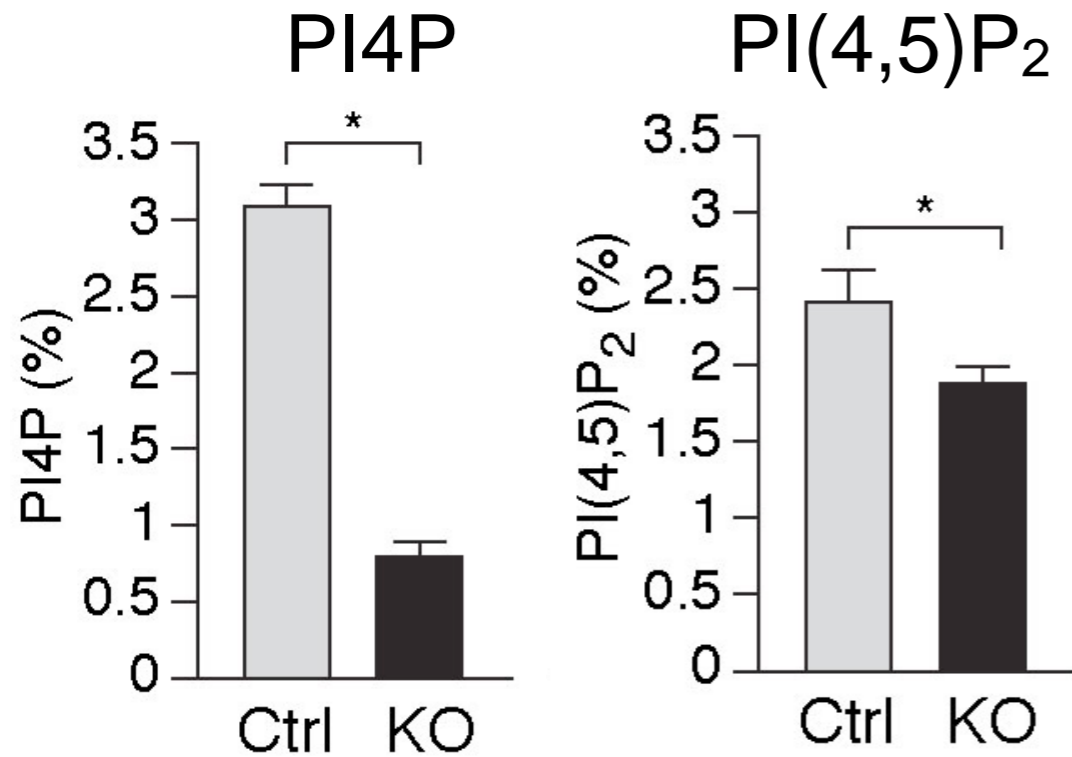


## Disruption of F-actin

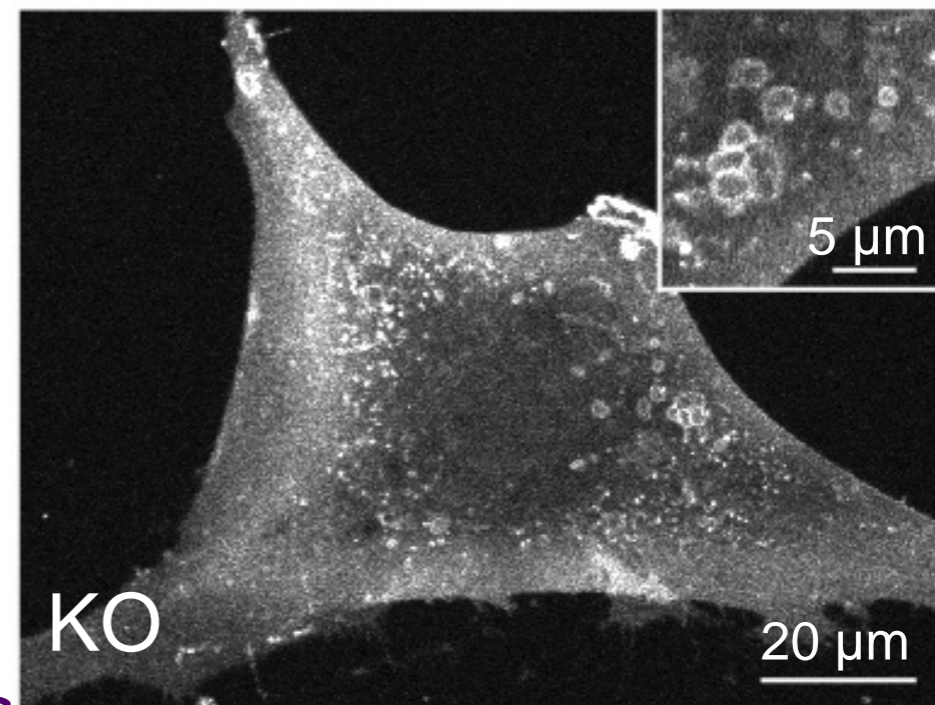
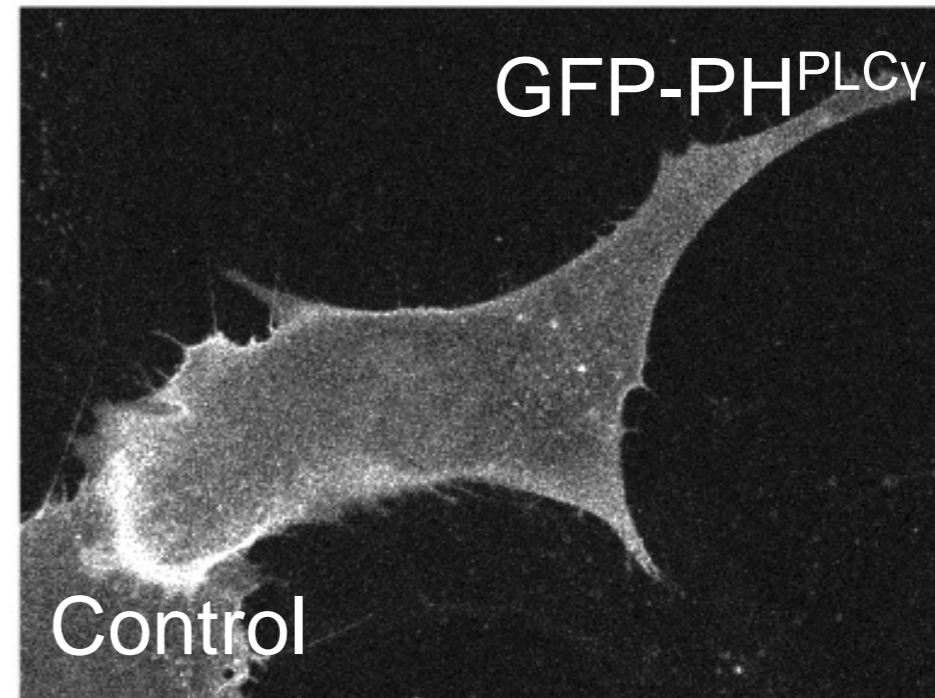
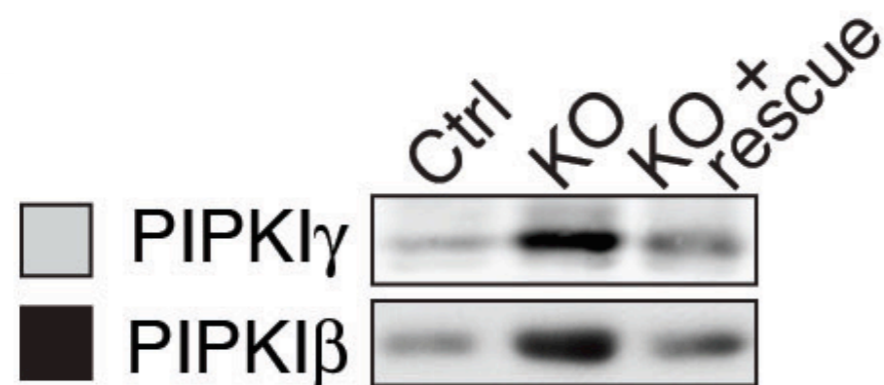


Phalloidin

# In PI4III $\alpha$ KO MEFs the drop of PI(4,5)P<sub>2</sub> is much less robust than the loss of PI4P



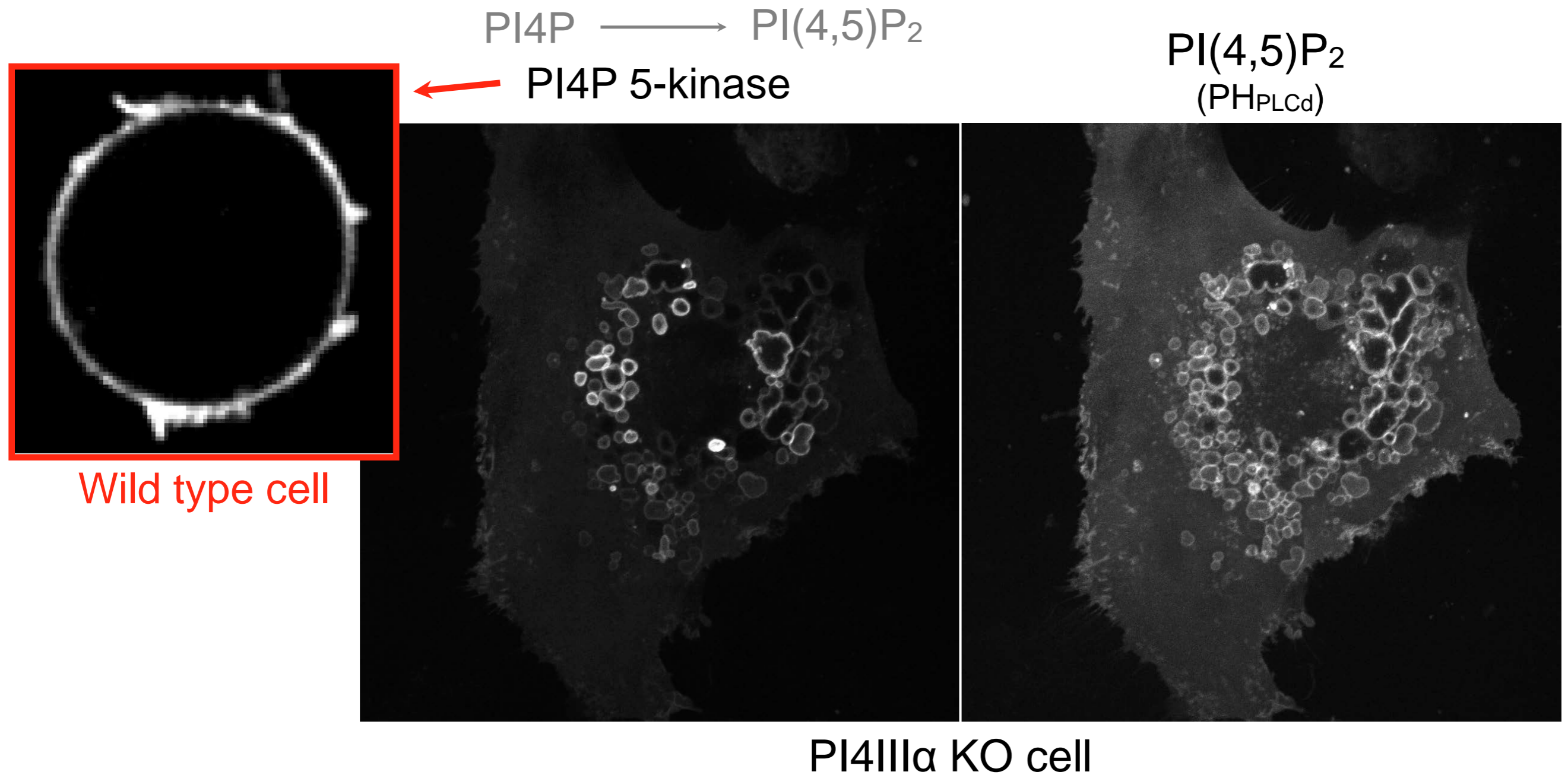
compensatory increase of PI4P 5-kinases  
(Type 1 PIP kinases)



PI(4,5)P<sub>2</sub> decreases at plasma membrane but accumulates on cytoplasmic vesicles

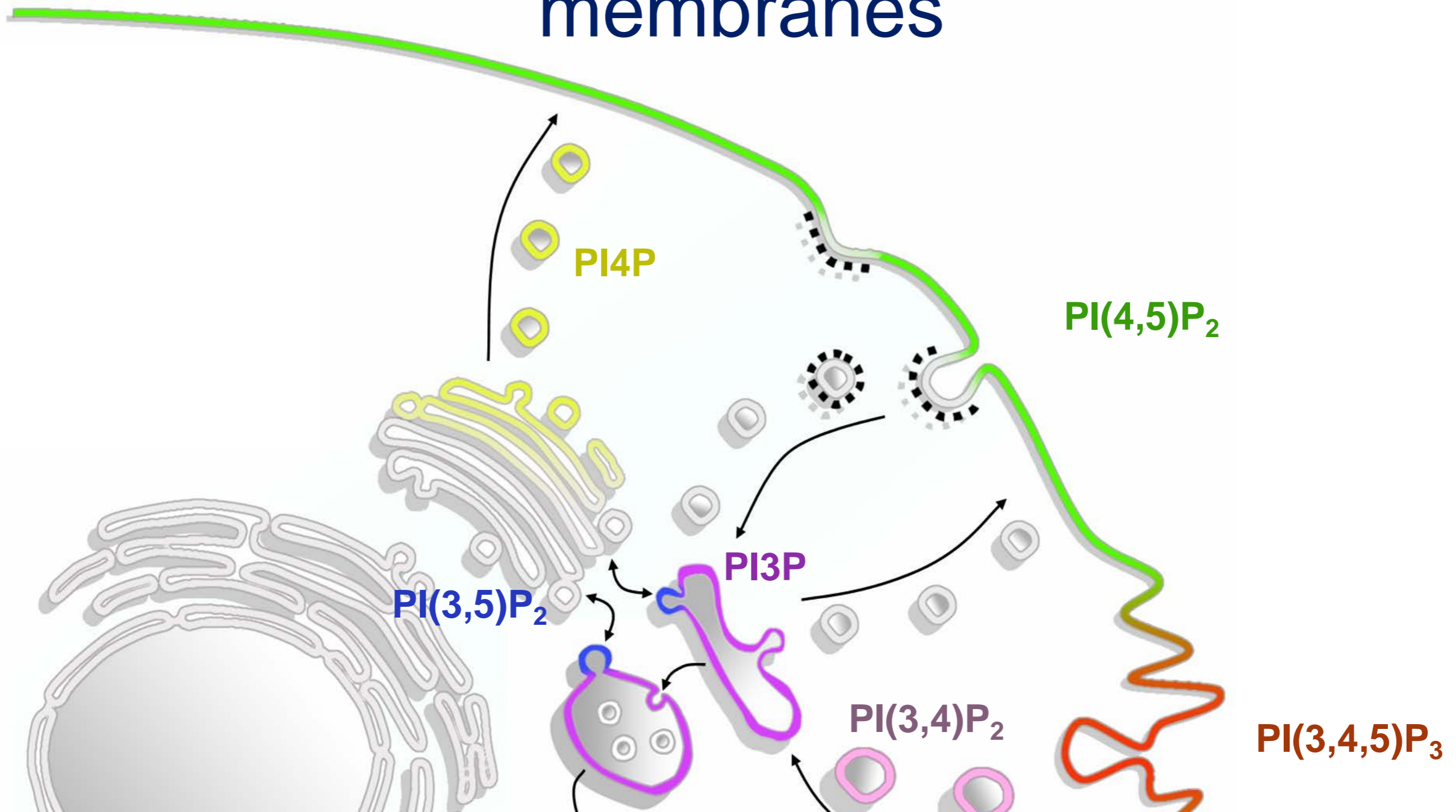


In PI4III $\alpha$  KO cells PI4P 5-kinases are localized on internal vesicles rather than at the plasma membrane



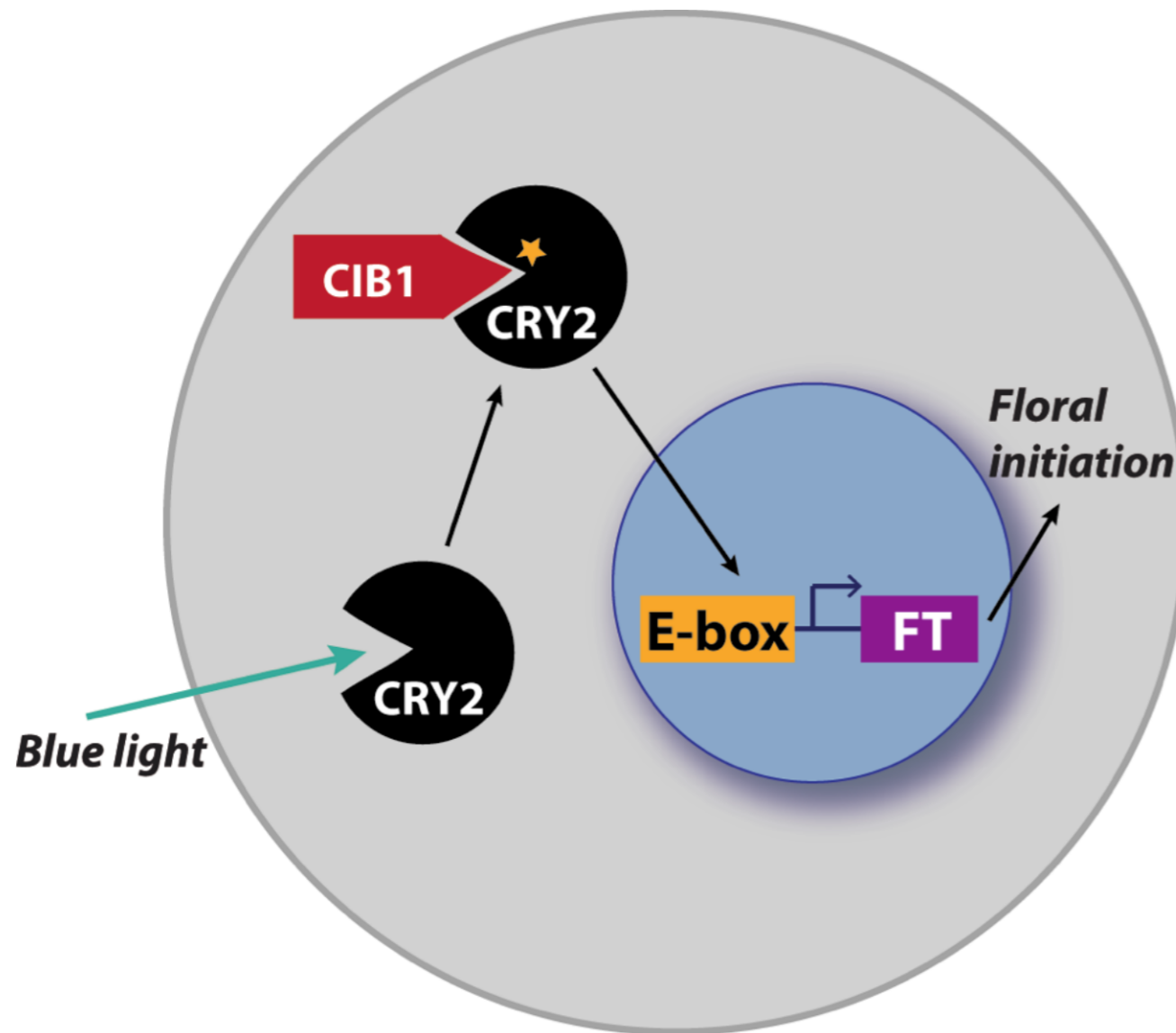


# A phosphoinositide code for cellular membranes



Can we by pass the problems intrinsic to genetic studies  
*? long-term nature -> adaptive/compensatory changes*

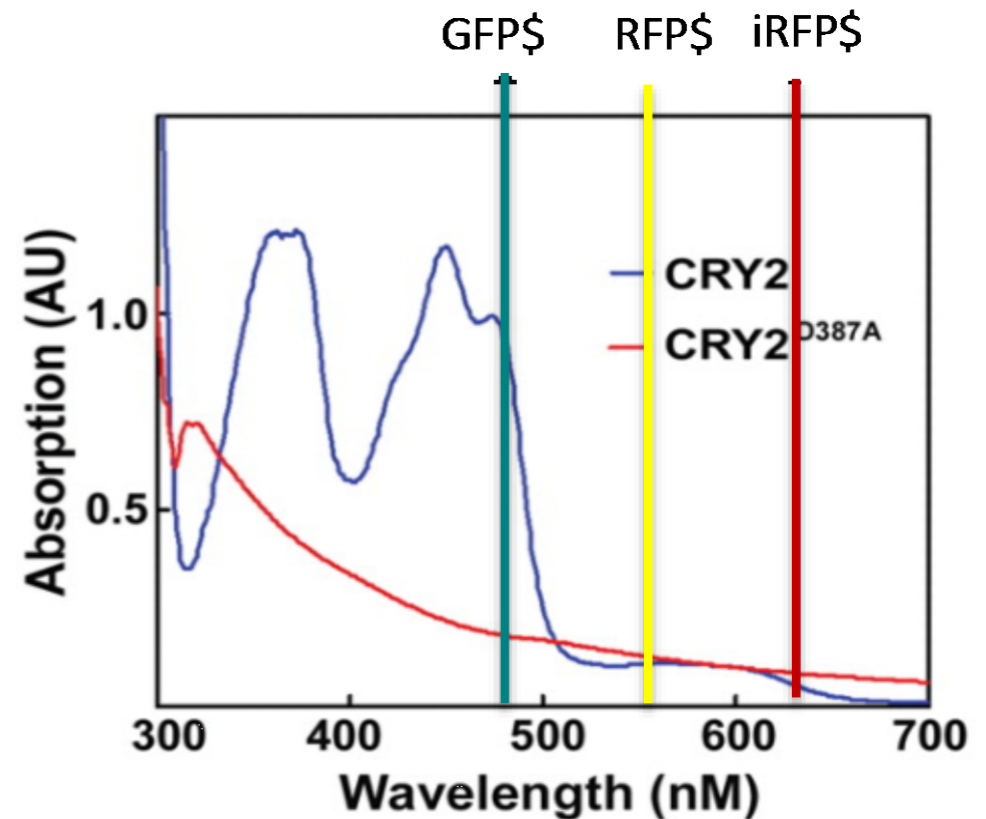
# Light-induced protein heterodimerization



cryptochrome = blue light receptor  
CIB1 = transcription factor

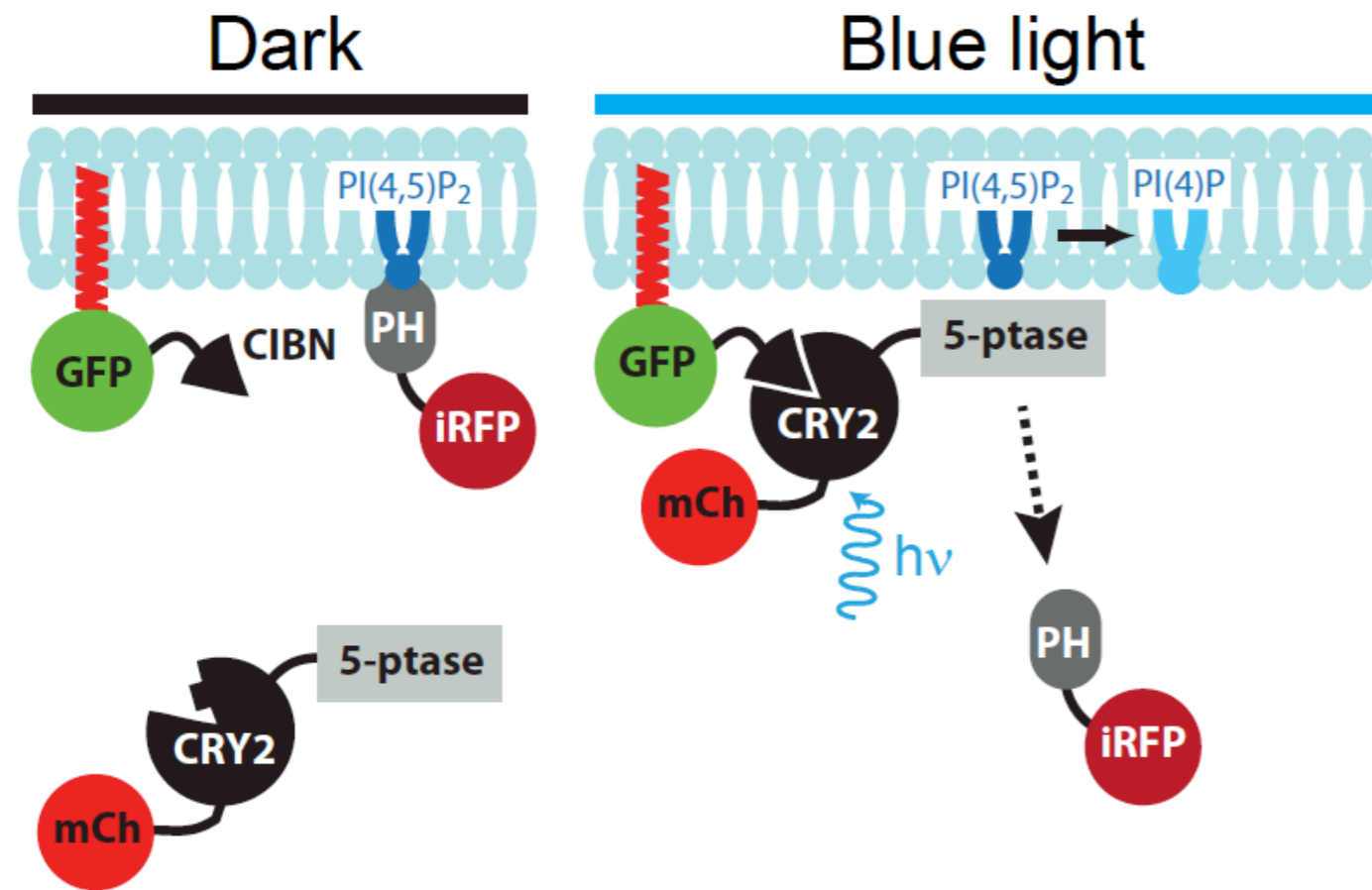
Cryptochromes regulate light-dependent transcription in plants

## Cryptochrome



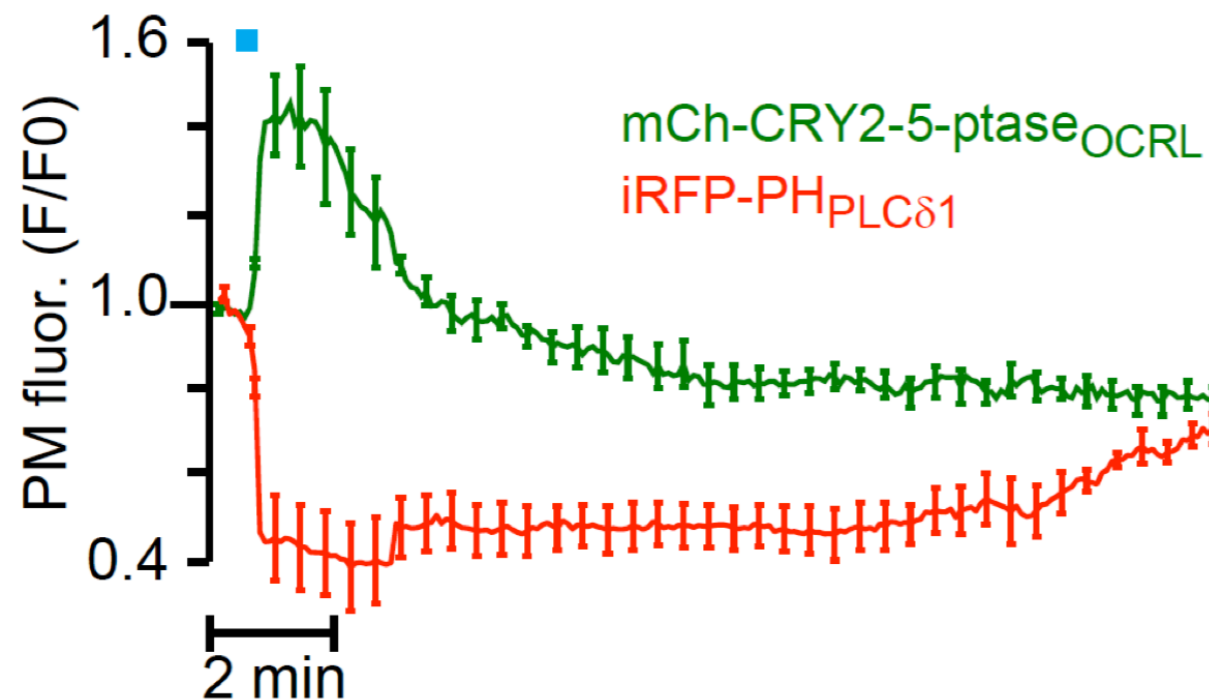
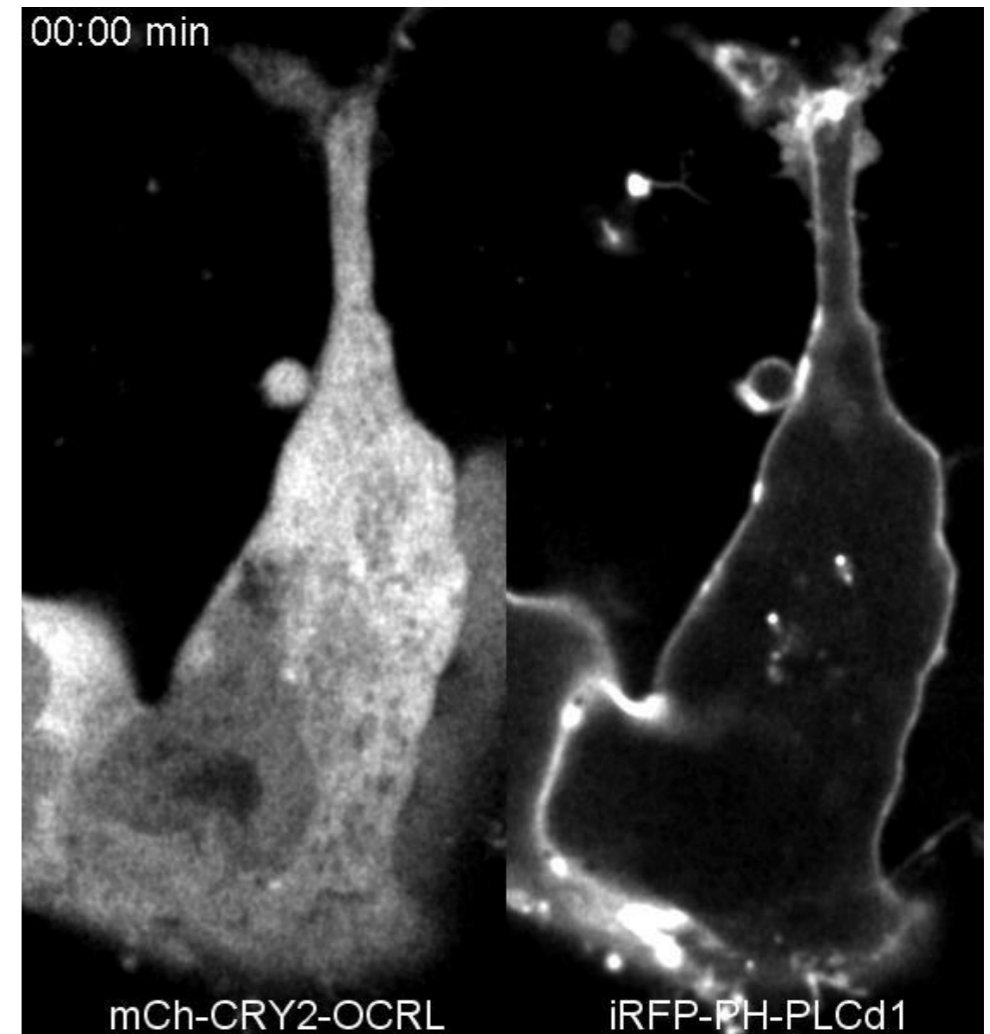
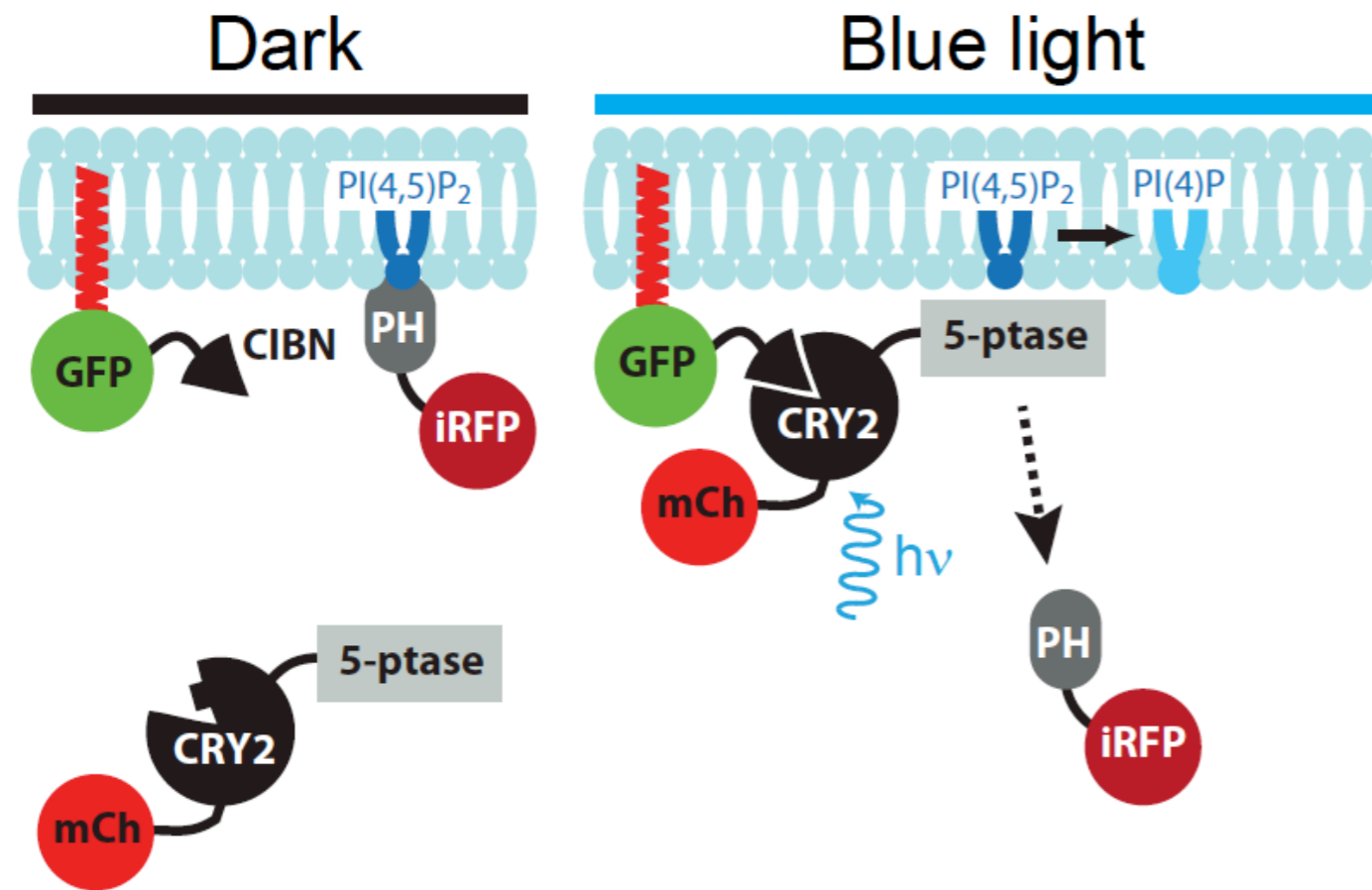
# Optogenetic depletion of PI(4,5)P<sub>2</sub> at the plasma membrane

(blue light-mediated recruitment of a PI(4,5)P<sub>2</sub> 5-phosphatase)



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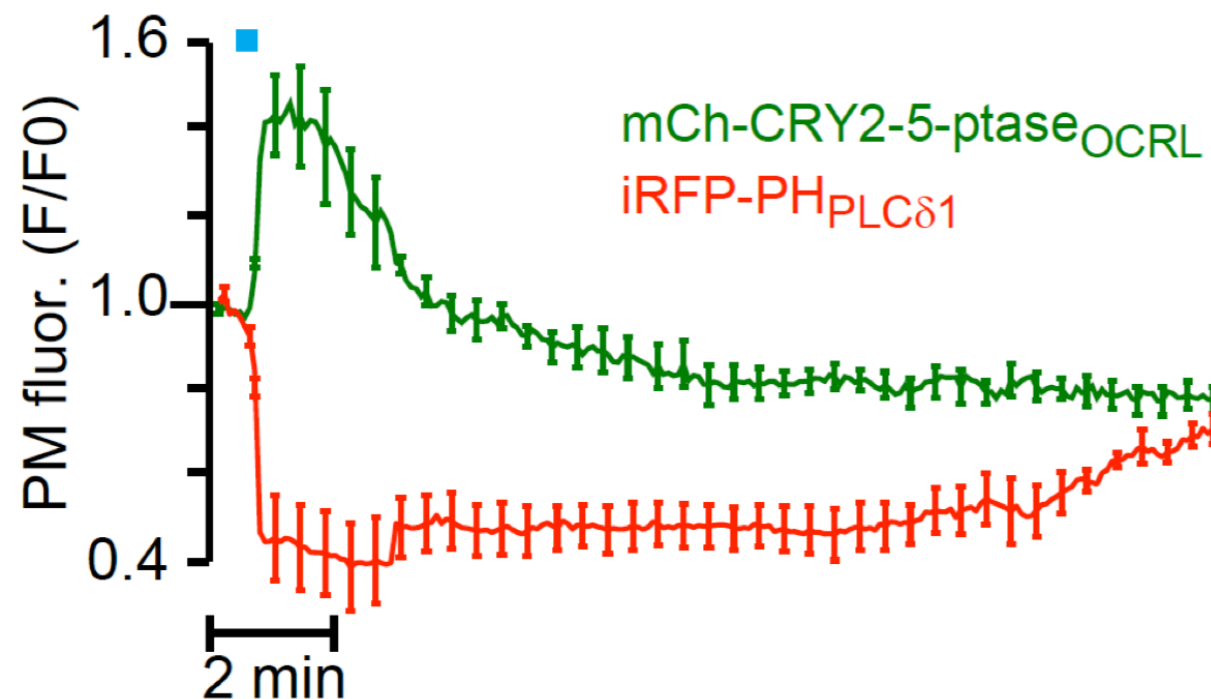
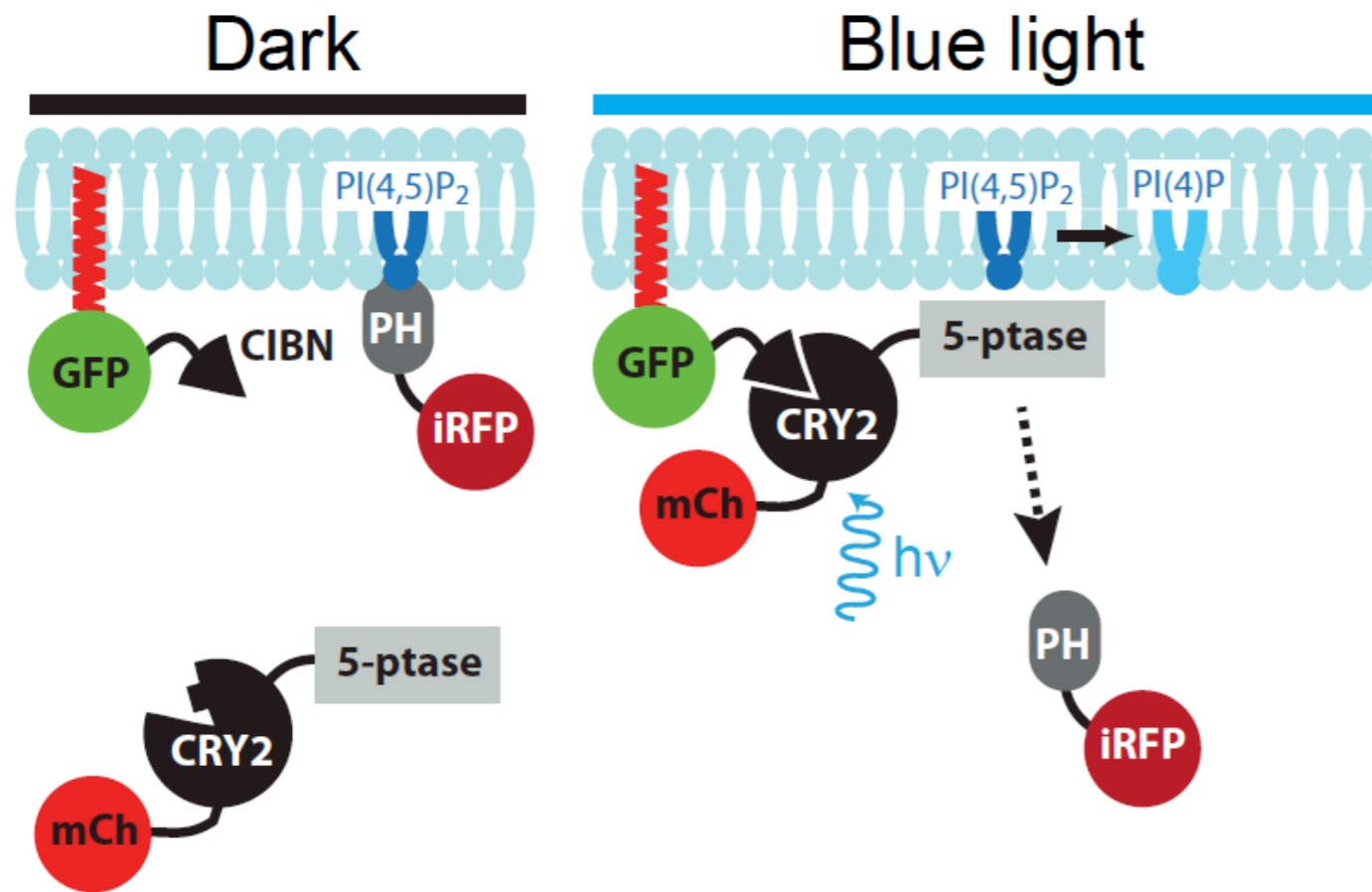
Recruitment  
 $t_{1/2} = 3.1 \pm 0.2$  s

Recovery (shedding)  
 $t_{1/2} = 6.8 \pm 1$  min

**Movie**

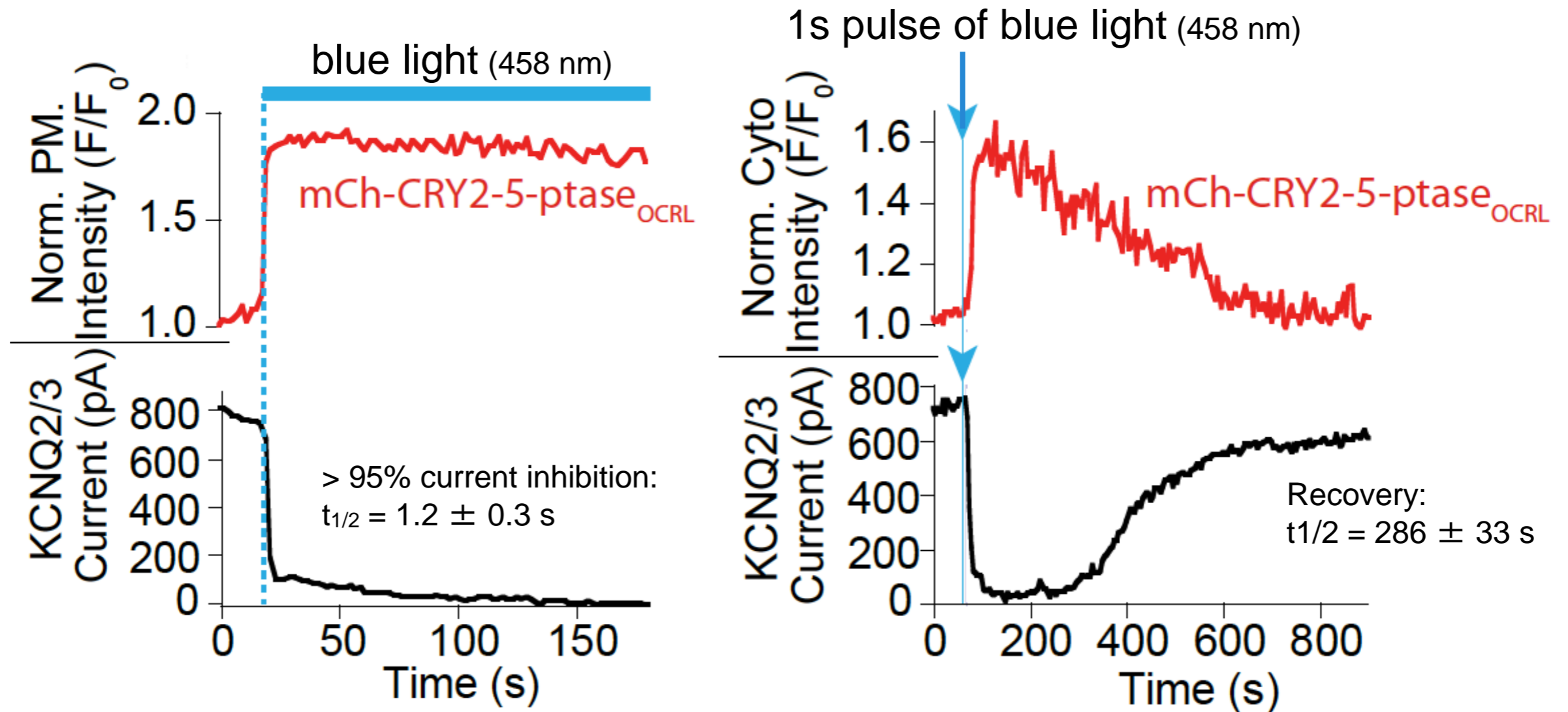


# PI(4,5)P<sub>2</sub> hydrolysis upon blue light-induced inositol 5'-phosphatase recruitment



# Loss of K<sup>+</sup> currents upon blue light-induced inositol 5'-phosphatase recruitment

KCNQ2/3 (Kv7.2/7.3) channels  
whole cell currents



Eamonn Dickson, Bertil Hille

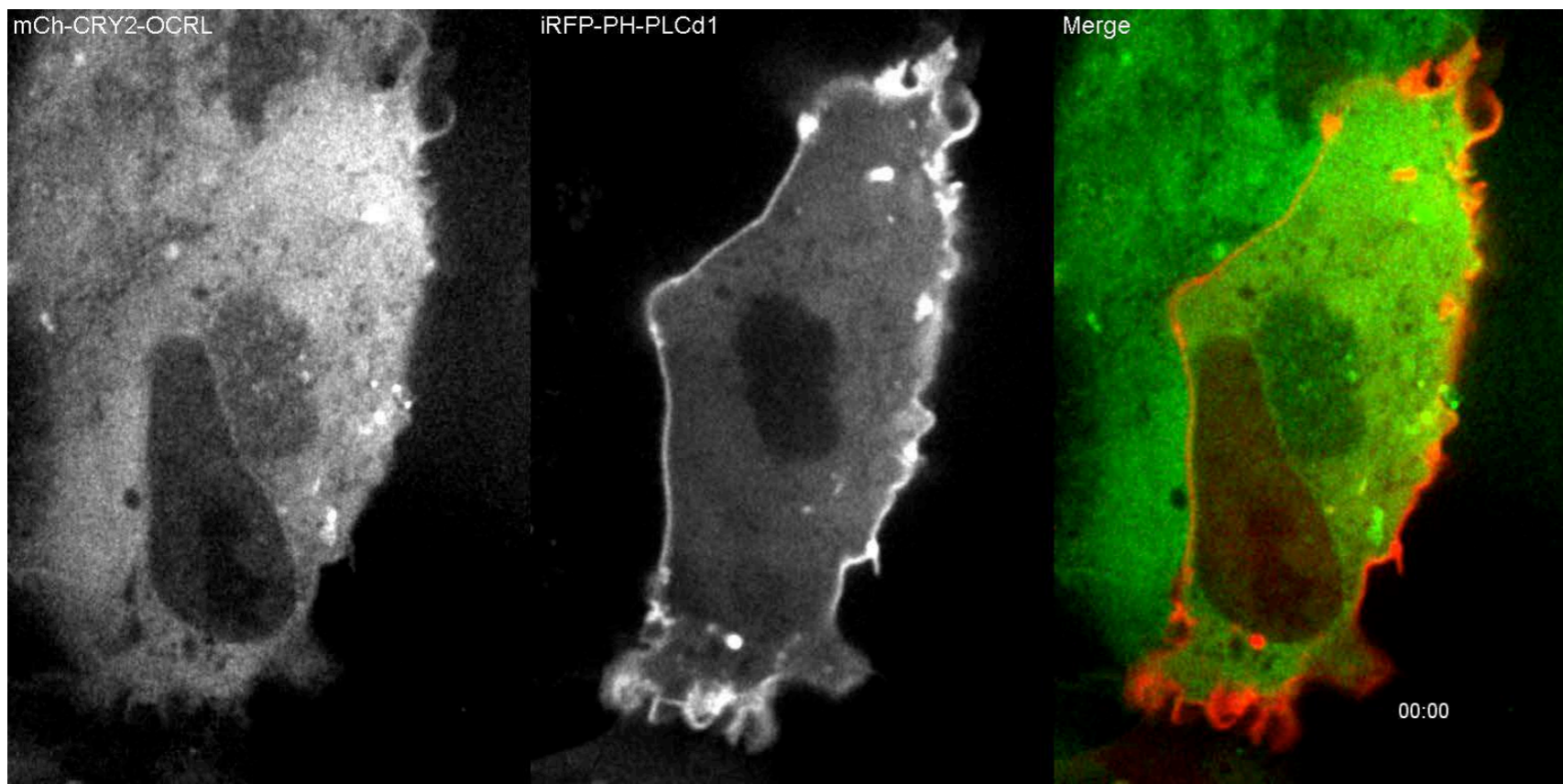
tsA-201 cells

Idevall-Hagren et al. PNAS 2012

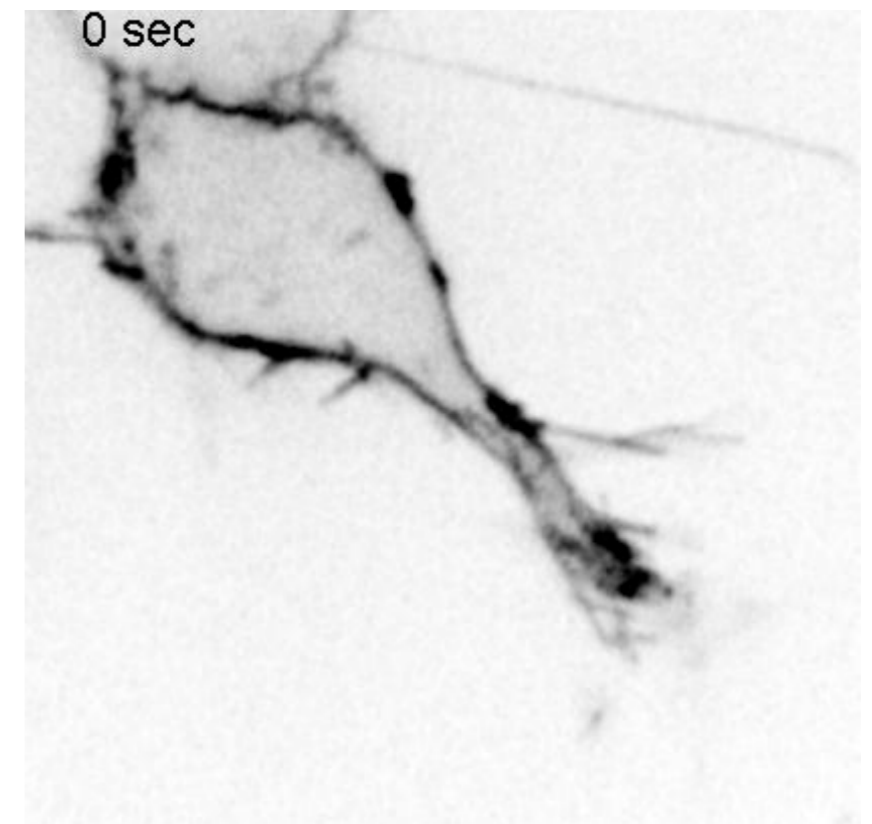
# Local blue light-illumination produces local effects and opposite effects at distant sites

Focal PI(4,5)P<sub>2</sub> depletion

**Movie**



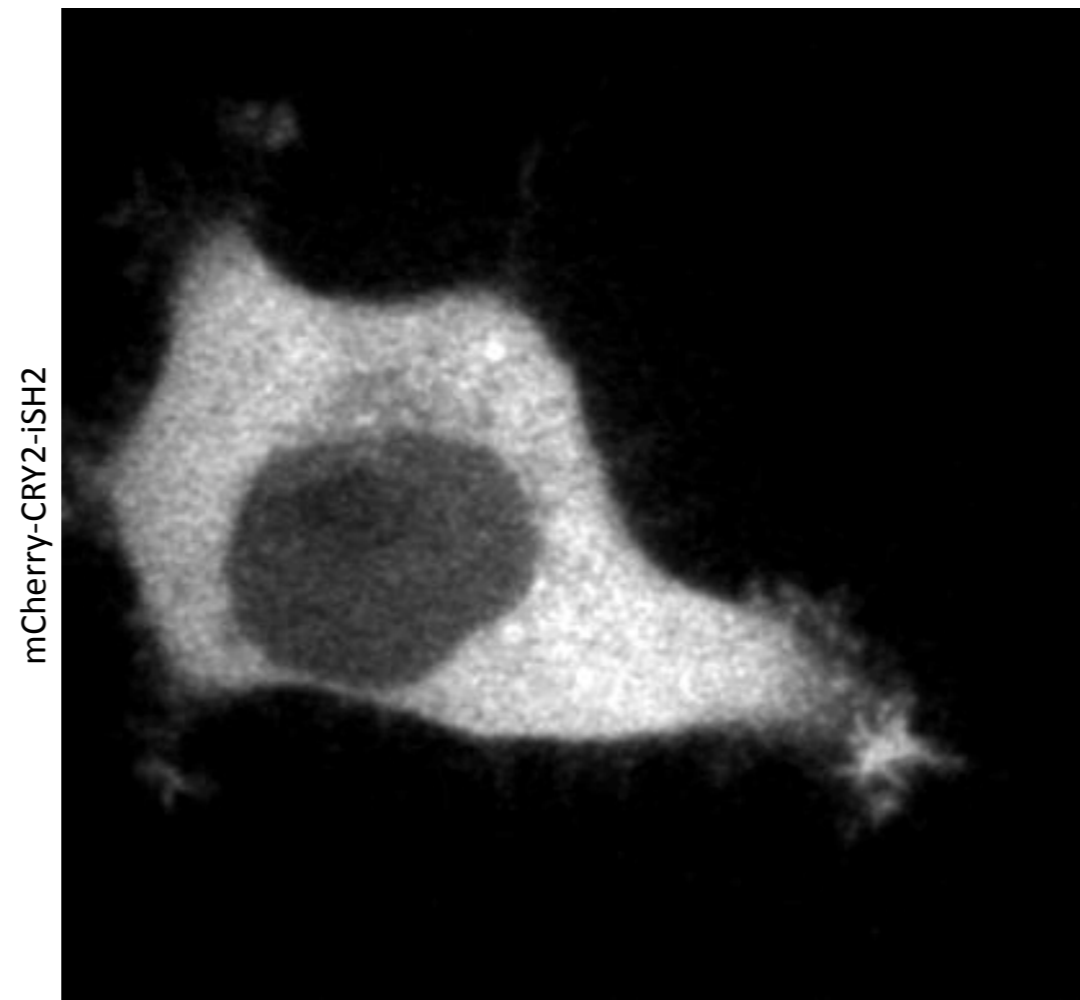
COS-7 cell



PC12 cells  
**Movie**



# Light-induced recruitment of PI 3-kinase to the plasma membrane and induction of ruffling

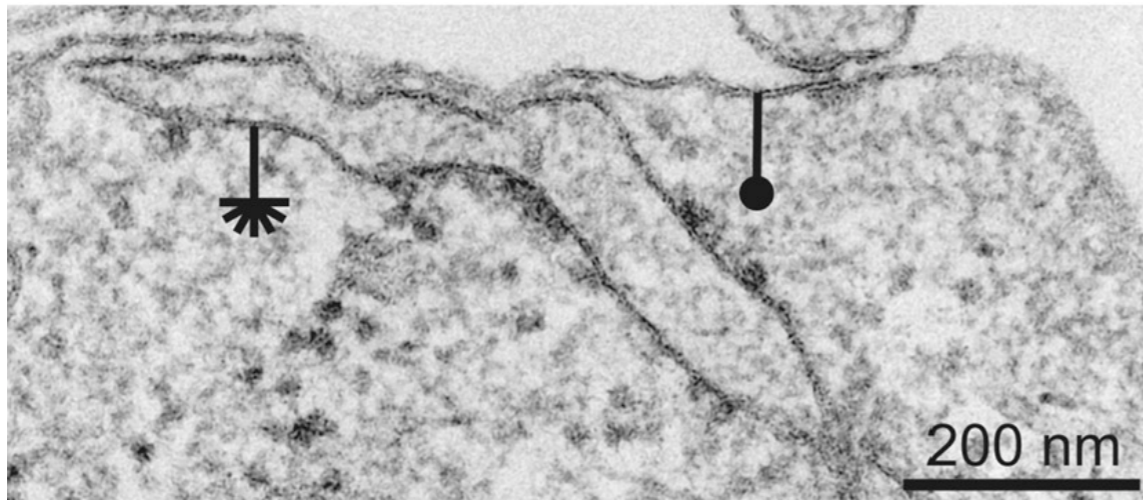


COS-7 cells

**Movie**

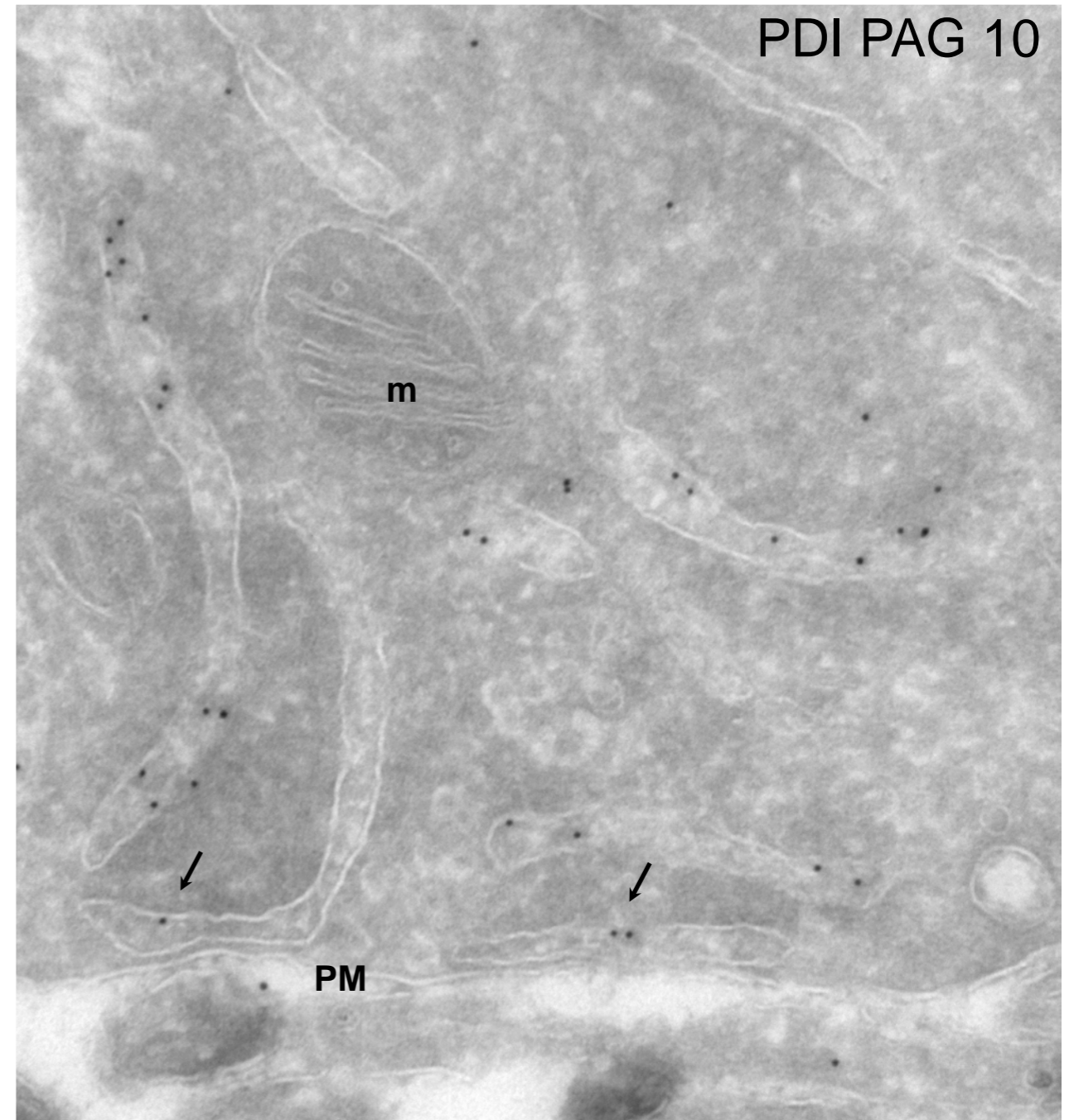
**Using optogenetics to discover new biology**

# ER-plasma membrane contacts



From Orci

fibroblast

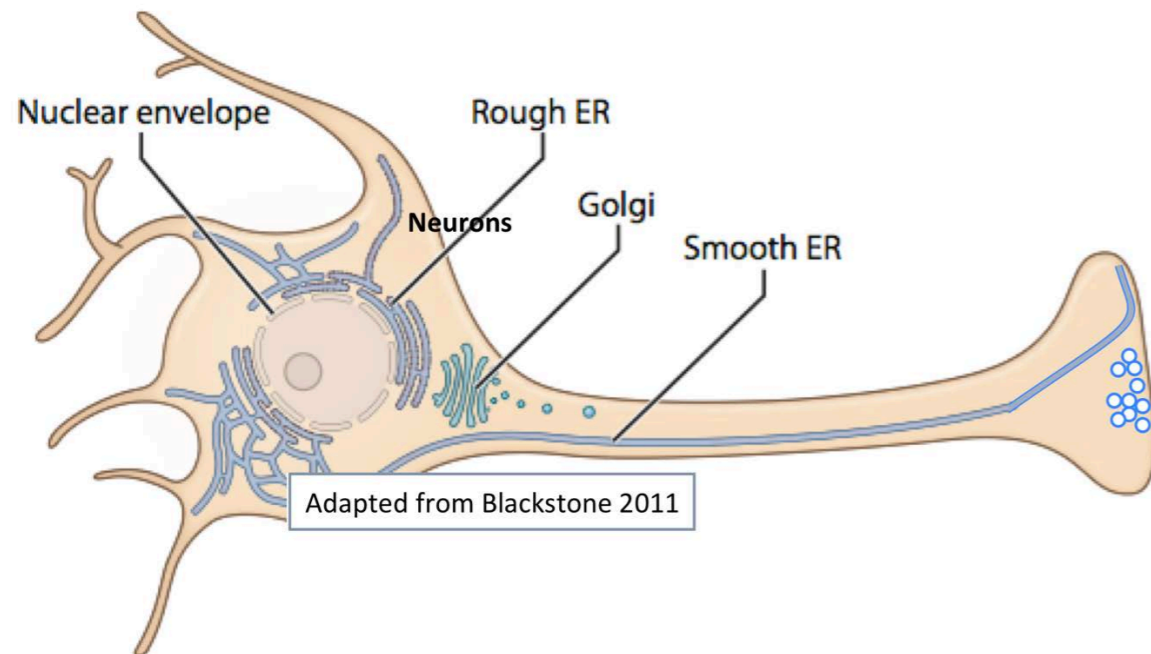


PDI PAG 10

m

PM

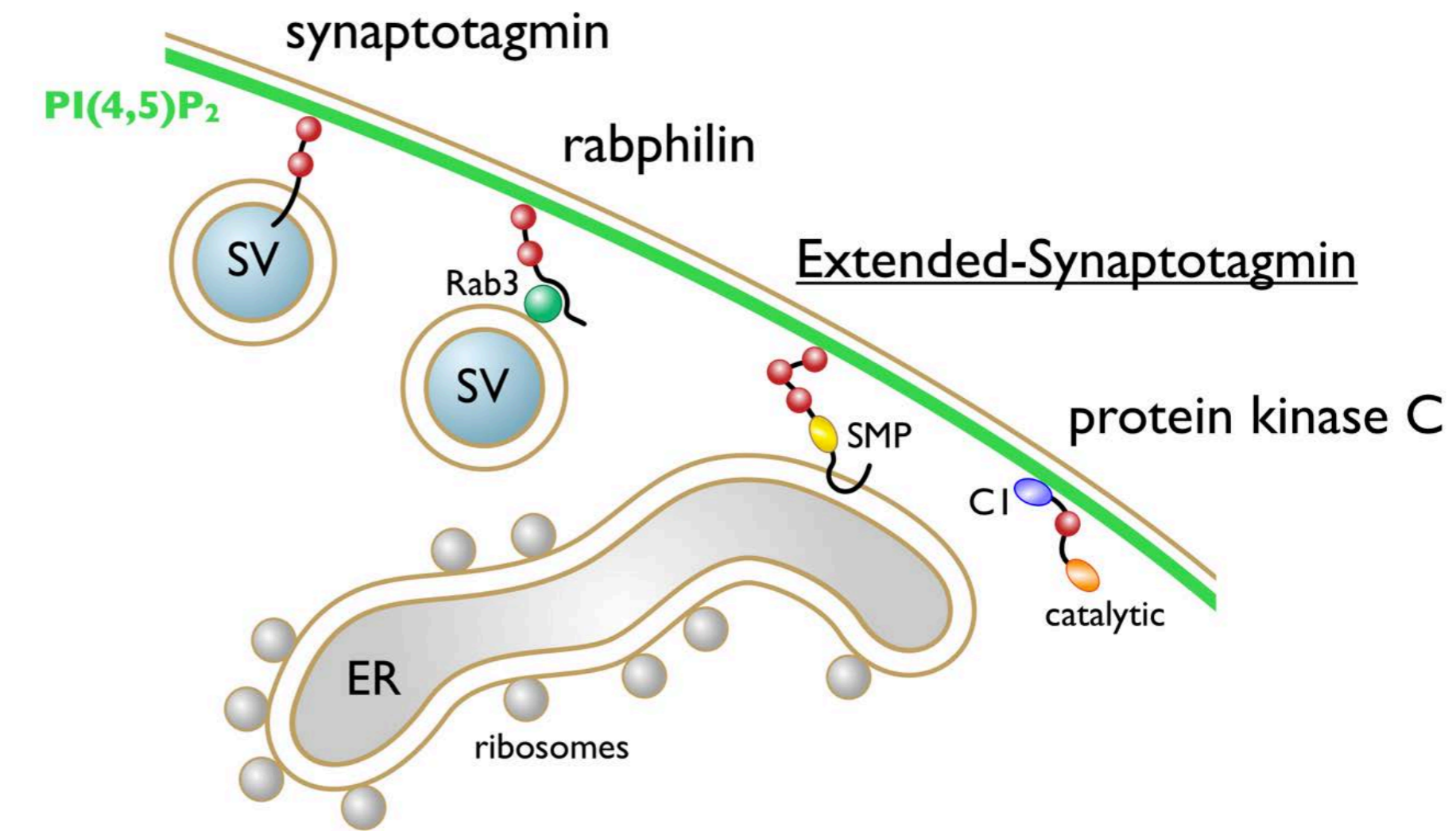
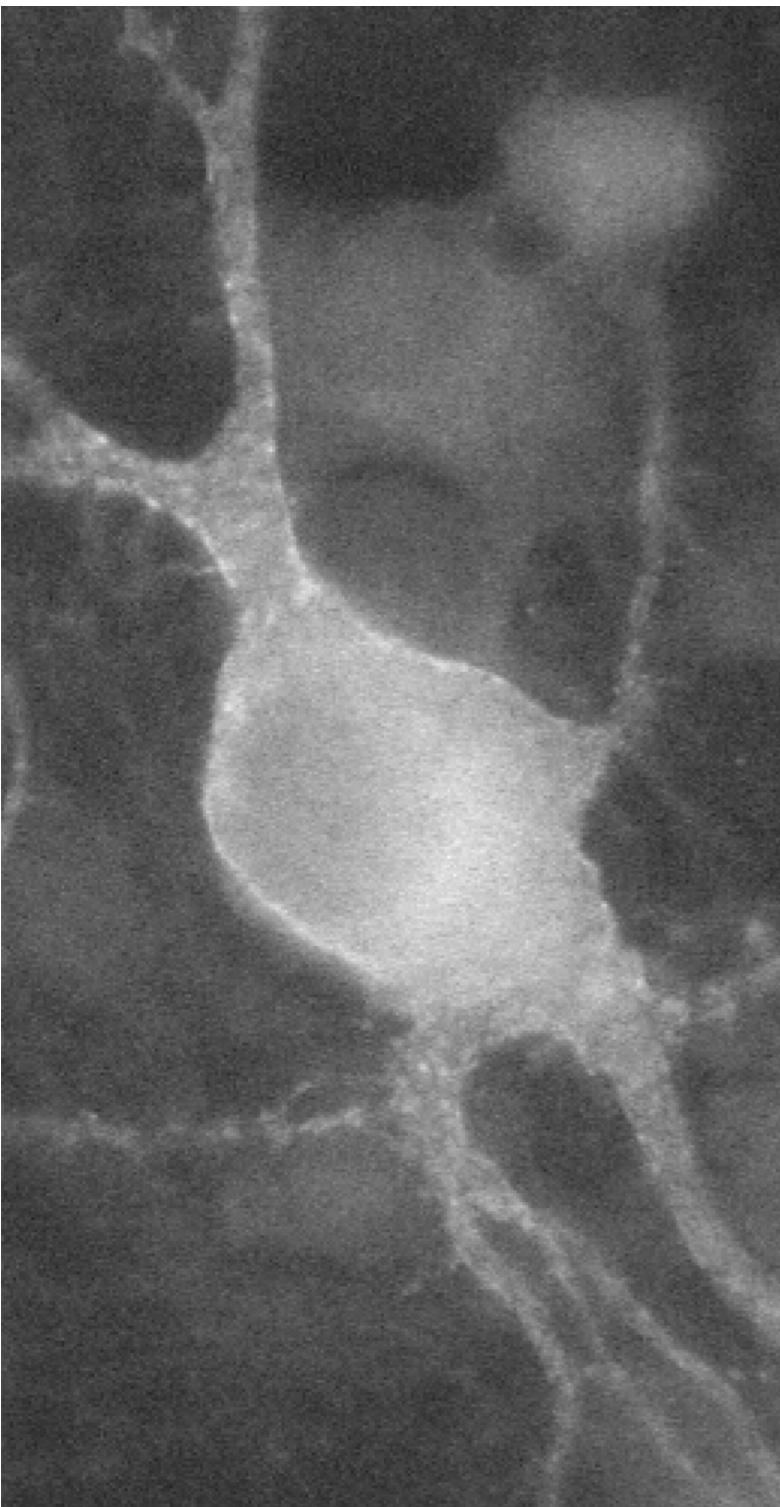
neuron



Adapted from Blackstone 2011



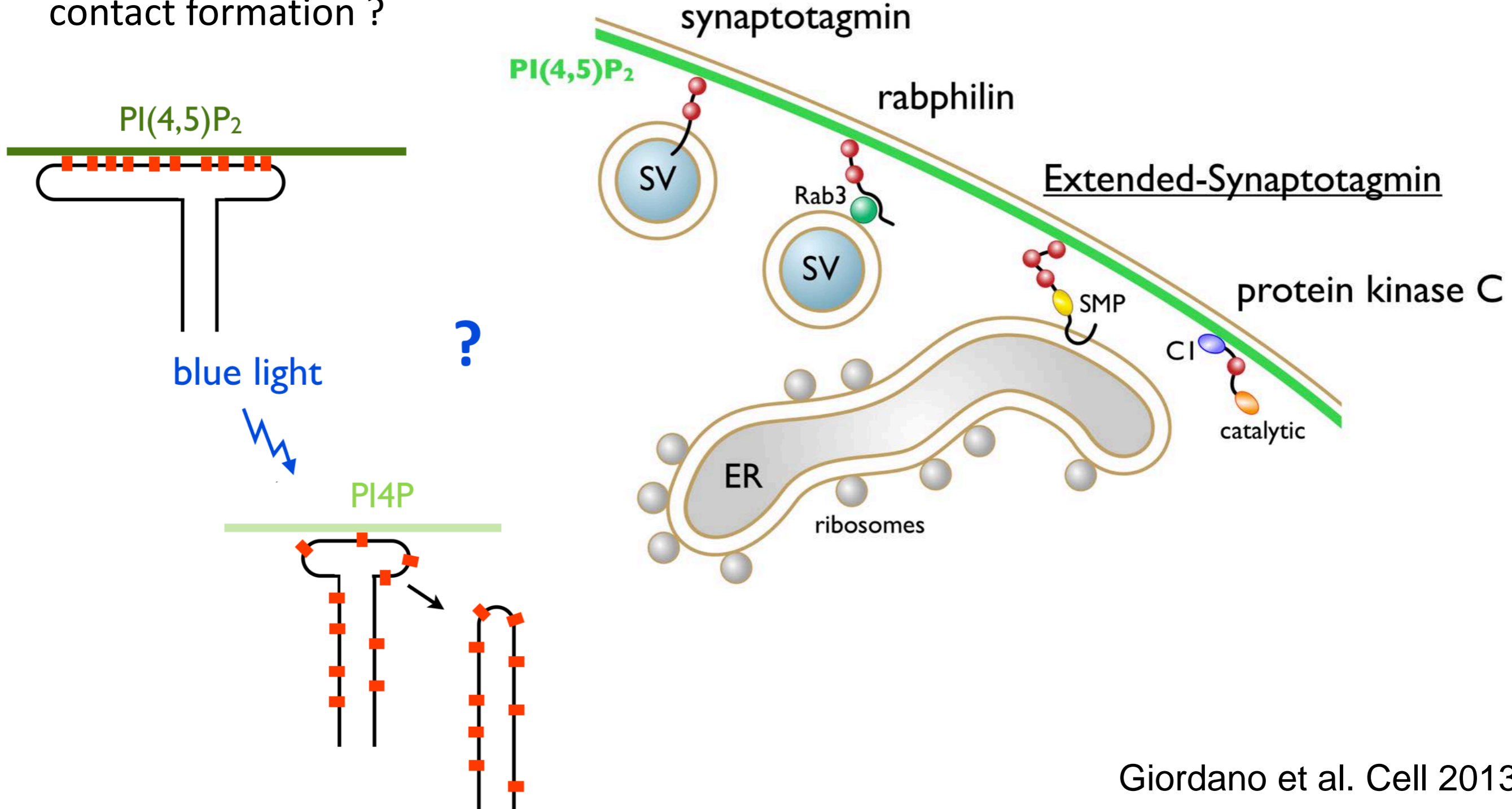
# The extended synaptotagmins: a family of proteins implicated in tethering the ER to the plasma membrane ?



E-Syt3 in neuron

# The extended synaptotagmins: a family of proteins implicated in tethering the ER to the plasma membrane ?

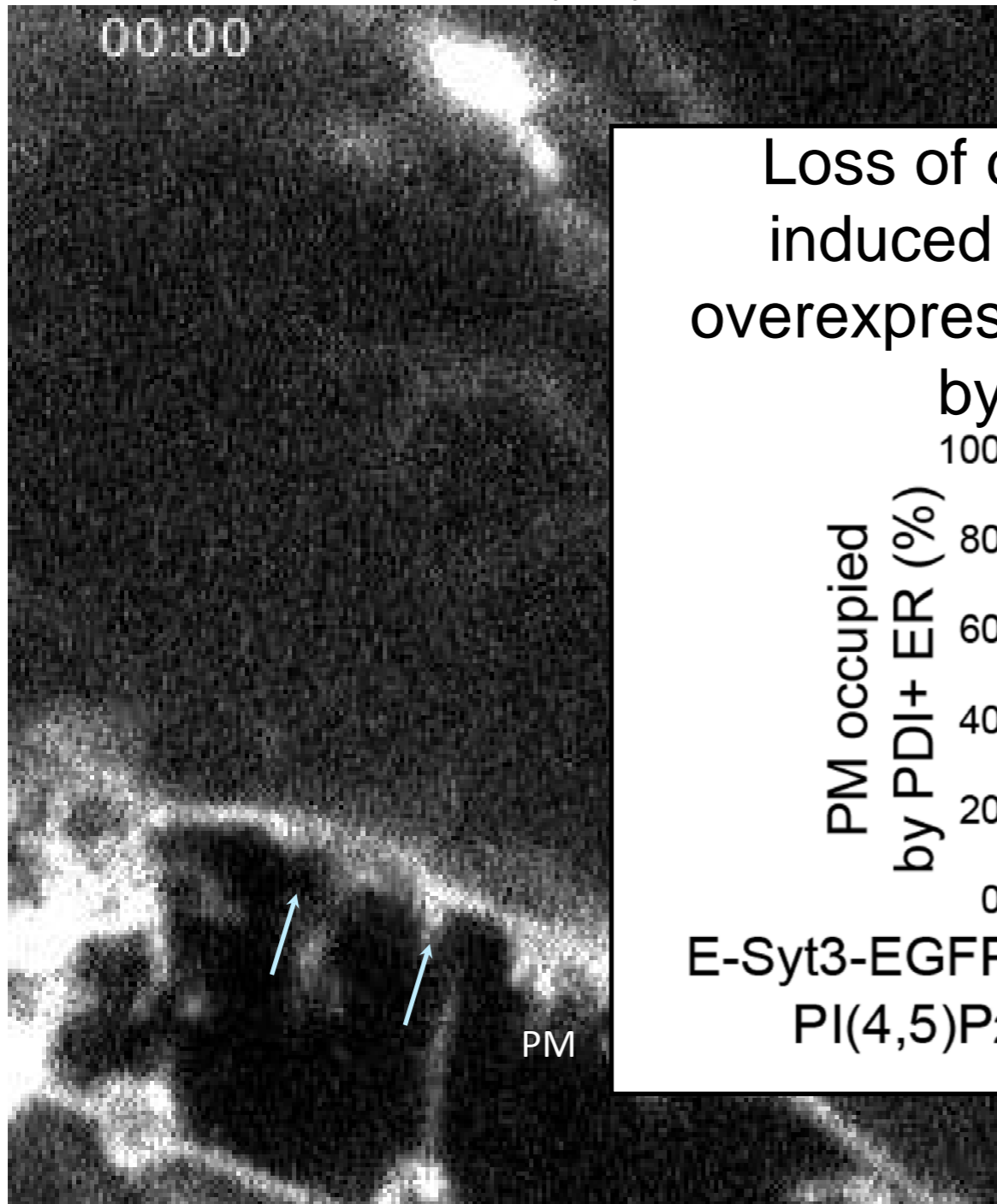
is PI(4,5)P<sub>2</sub> needed to contact formation ?



# Optogenetic depletion of PI(4,5)P<sub>2</sub> dissociates E-Syt3 from the plasma membrane

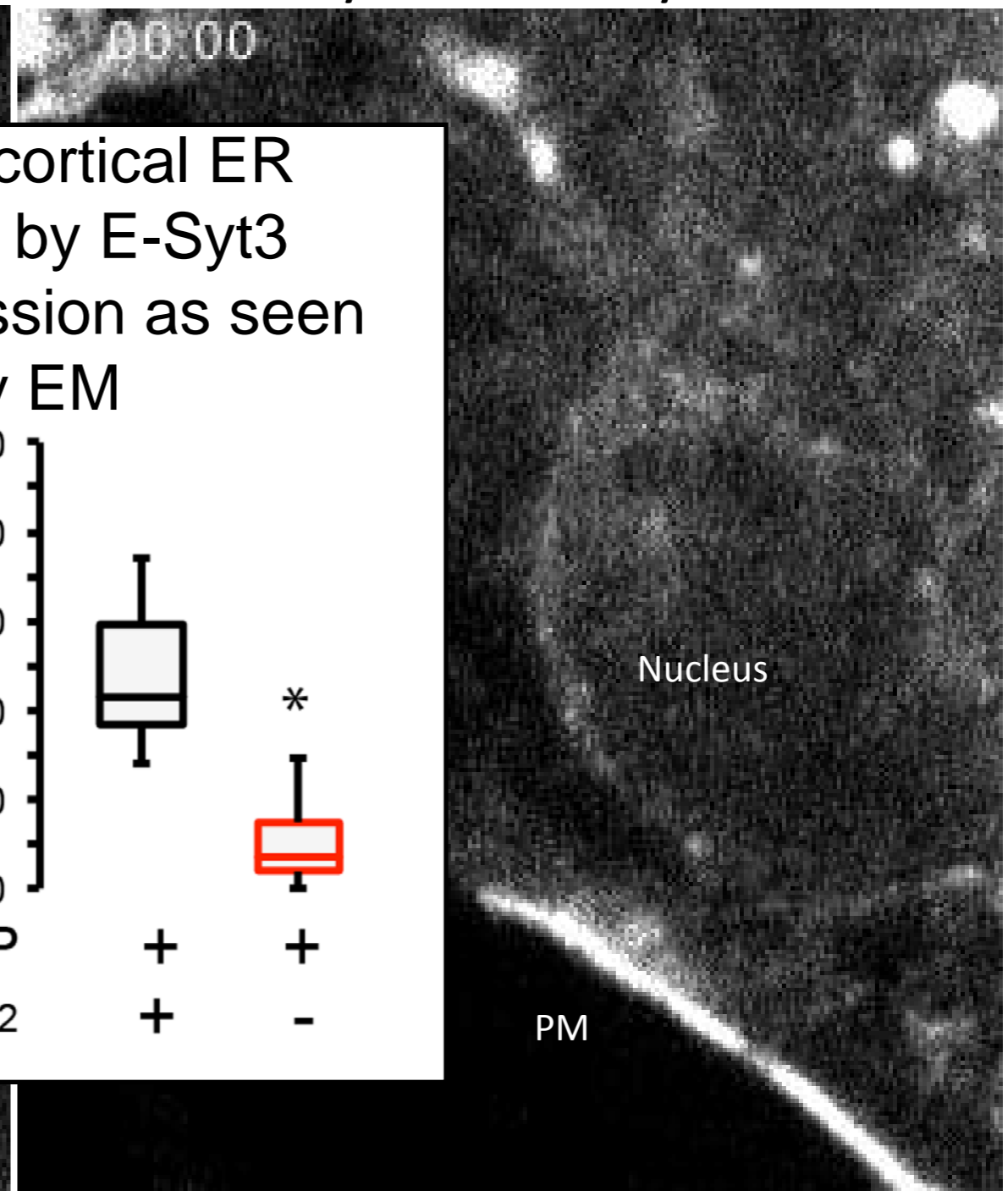
Movie

iRFP-PH-PLC $\delta$  [PI(4,5)P<sub>2</sub> biosensor]



Movie

E-Syt3-mCherry



Loss of cortical ER induced by E-Syt3 overexpression as seen

