



# Targeting the A-kinase Anchoring Function of PI3K $\gamma$ for Treating Cystic Fibrosis

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University of Torino  
Molecular Biotechnology Center  
Dept. of Molecular Biotechnology and Health  
Sciences - Torino (ITALY)



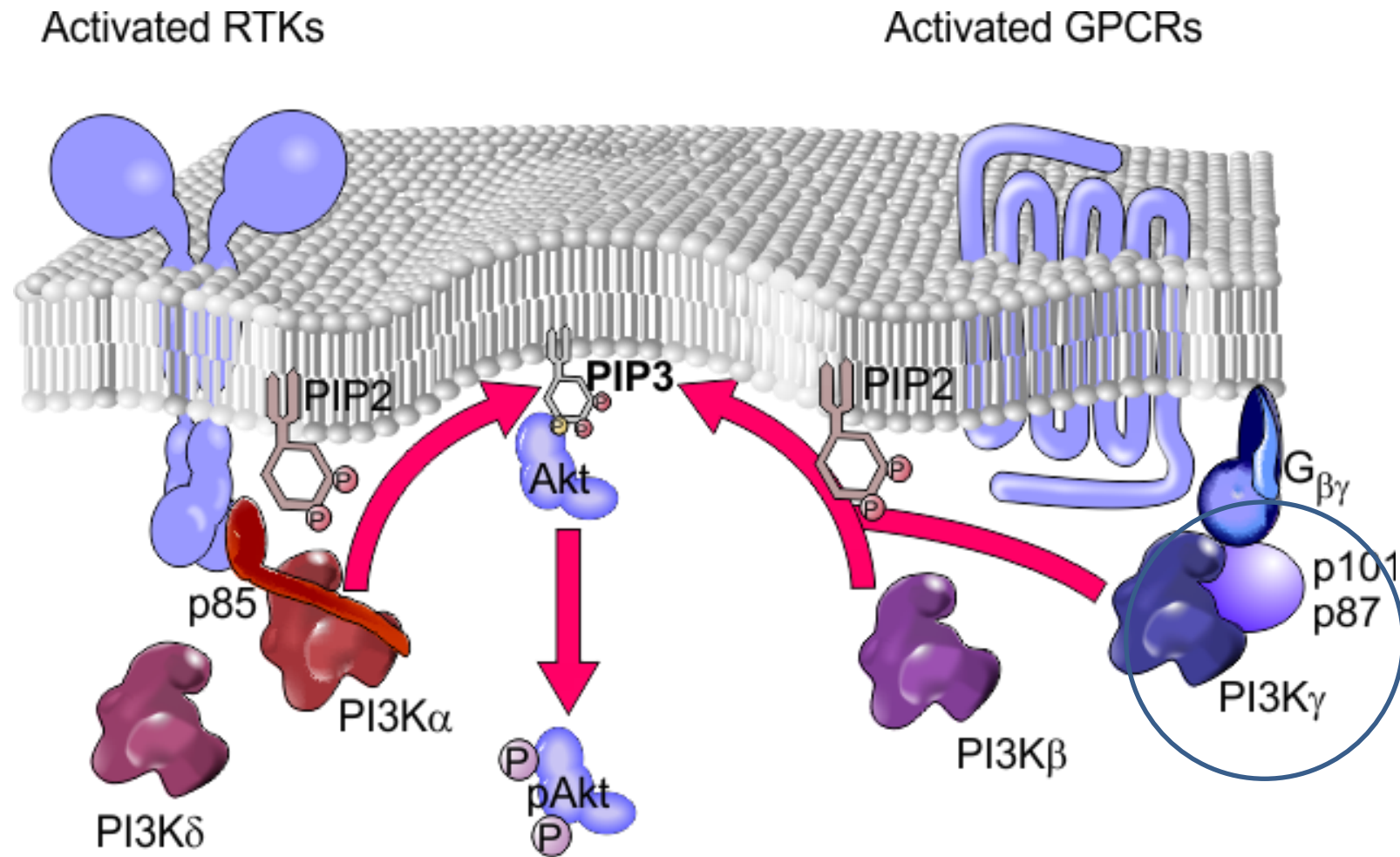
UNIVERSITÀ  
DEGLI STUDI  
DI TORINO

## **Disclosure**

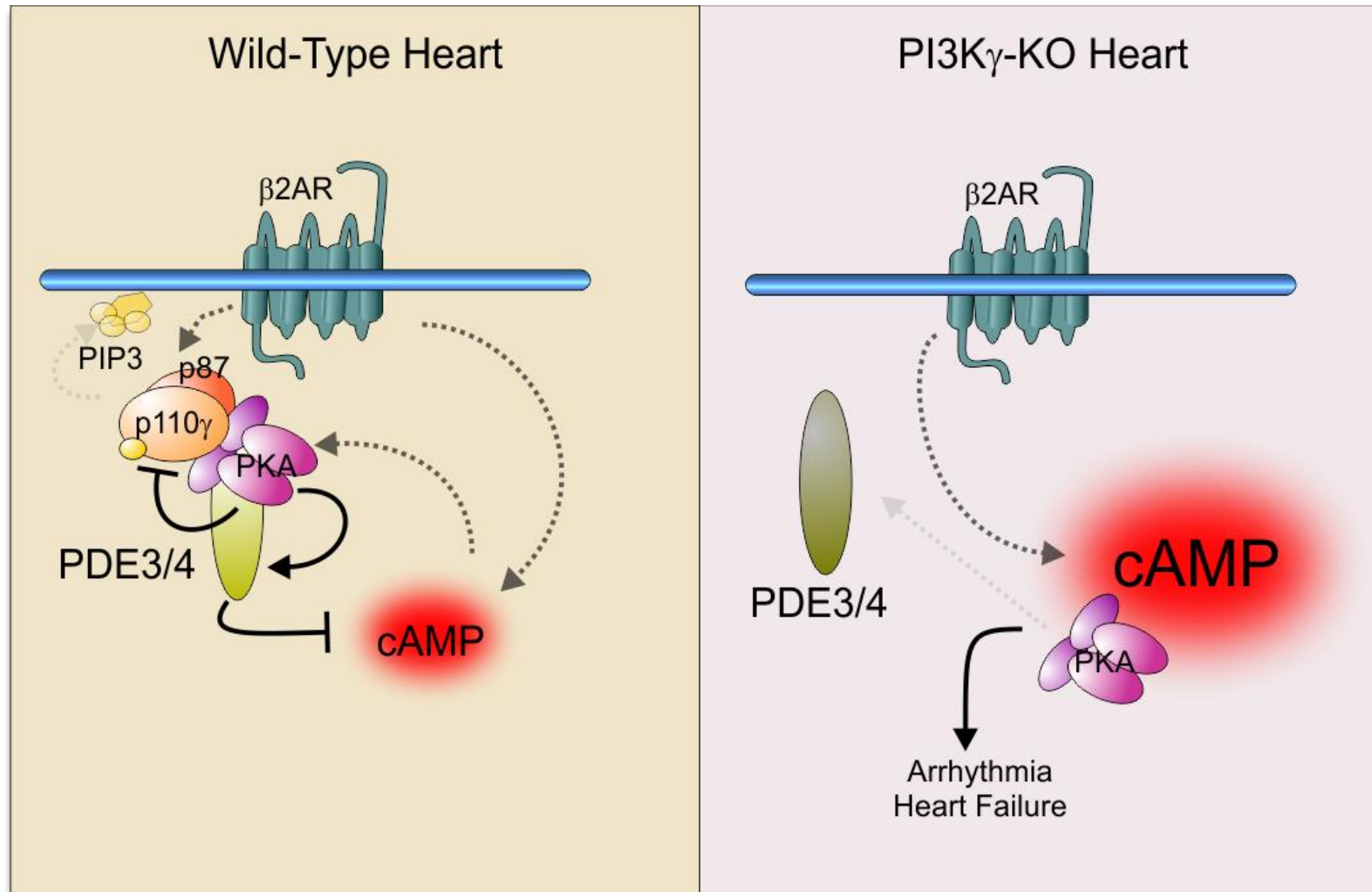
Co-founder and Board Member of Kither Biotech,  
a spin-off company focused on the development  
of the PI3K $\gamma$  Mimetic Peptide for the treatment of  
respiratory diseases



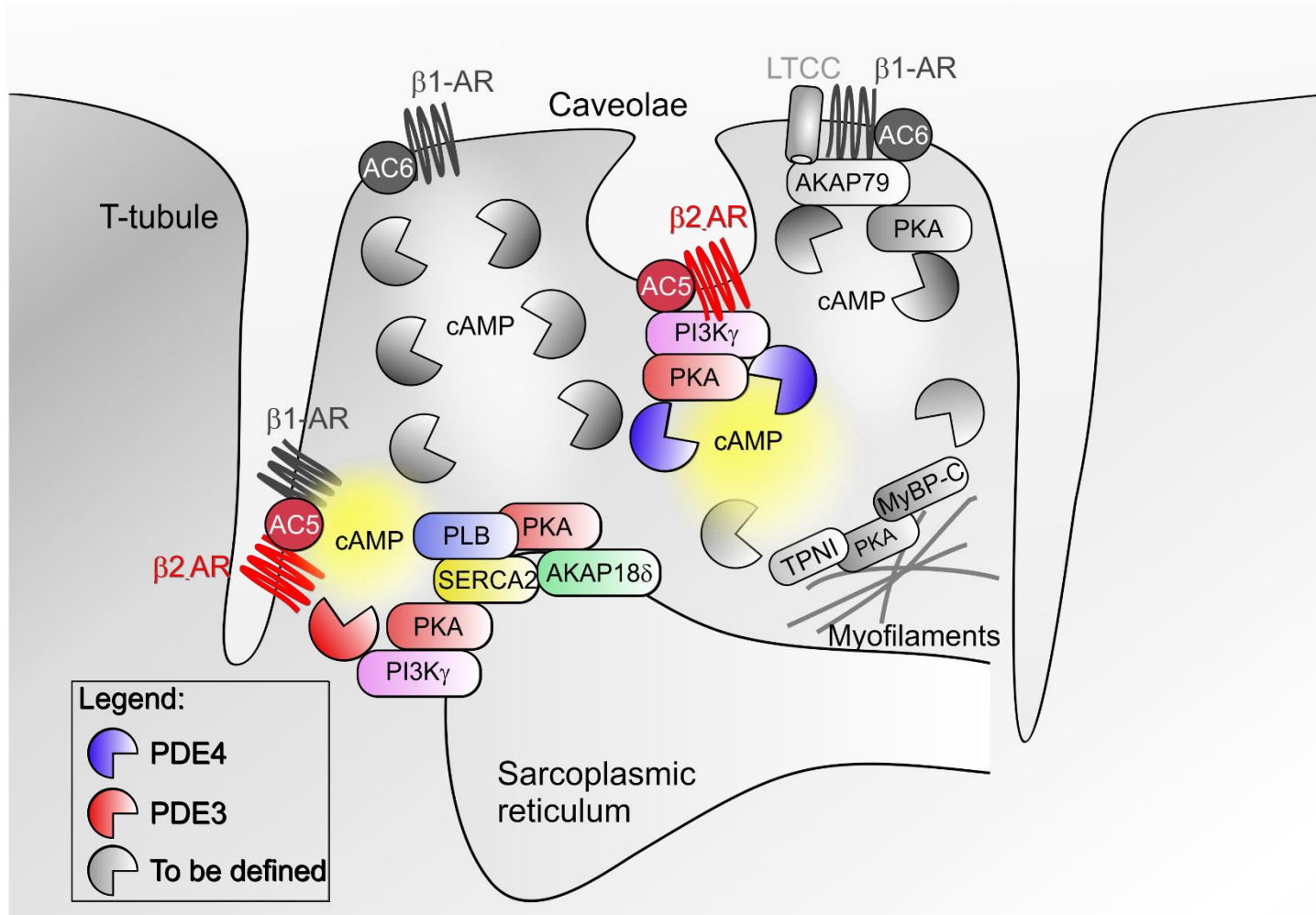
# Class I Phosphatidylinositol 3-kinases (PI3Ks)



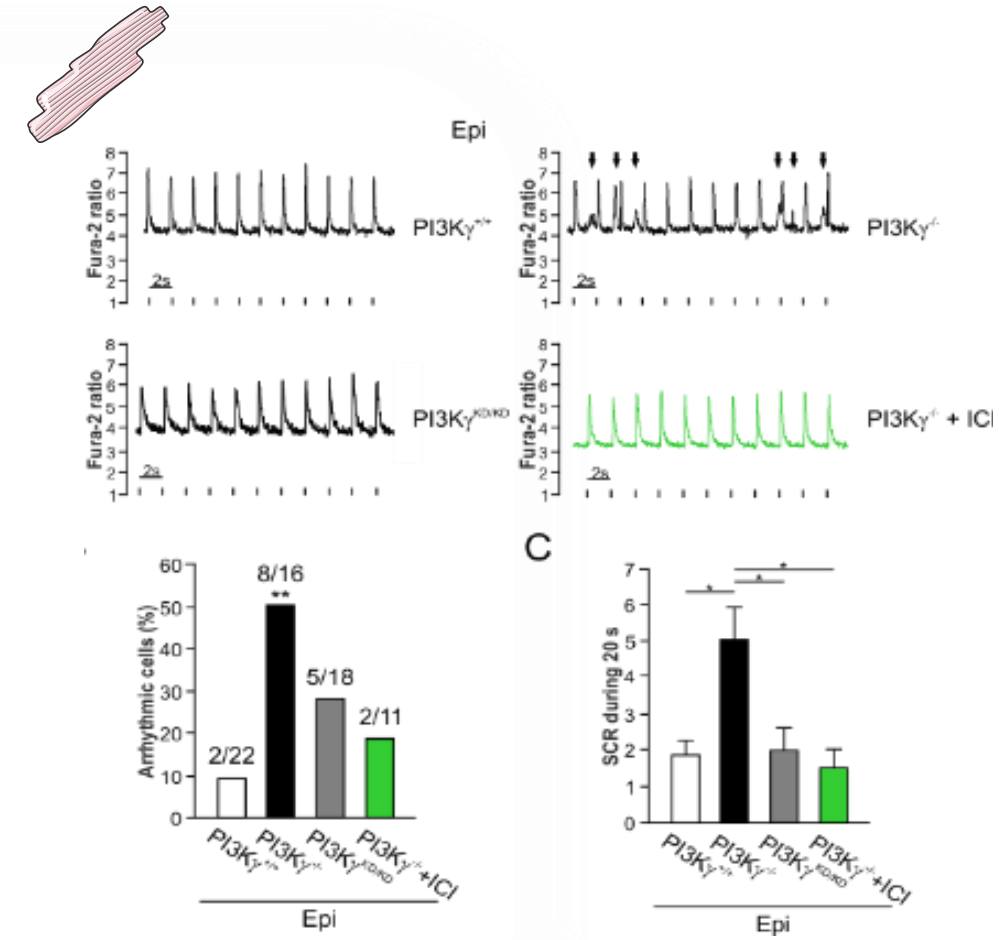
# The Kinase-independent Function of PI3K $\gamma$



# $\beta_2$ -AR-Induced Lethal Arrhythmias in PI3K $\gamma$ KO but not PI3K $\gamma$ KD Mice

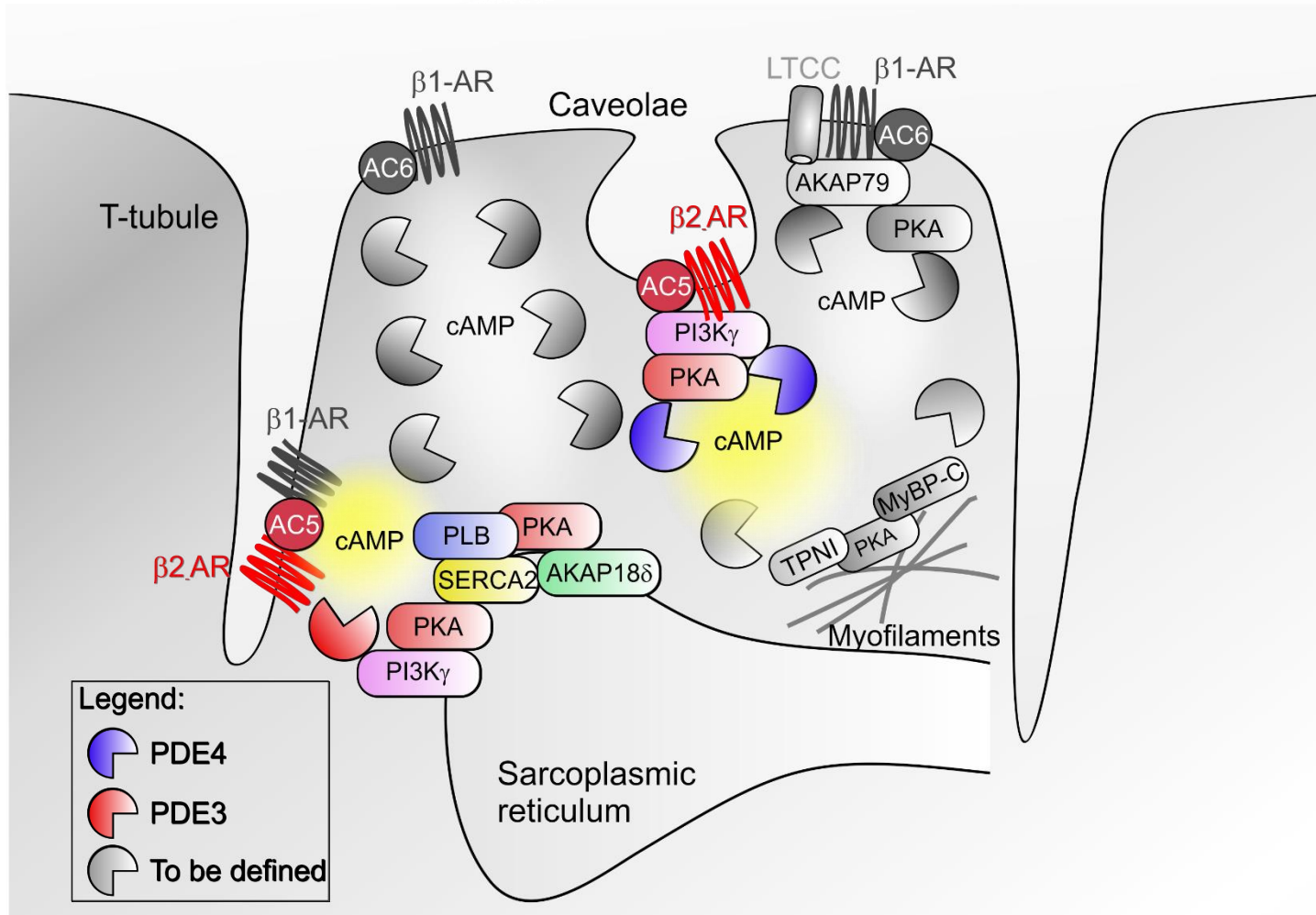


Ghigo & Mika, *J Mol Cell Cardiol.* 2019

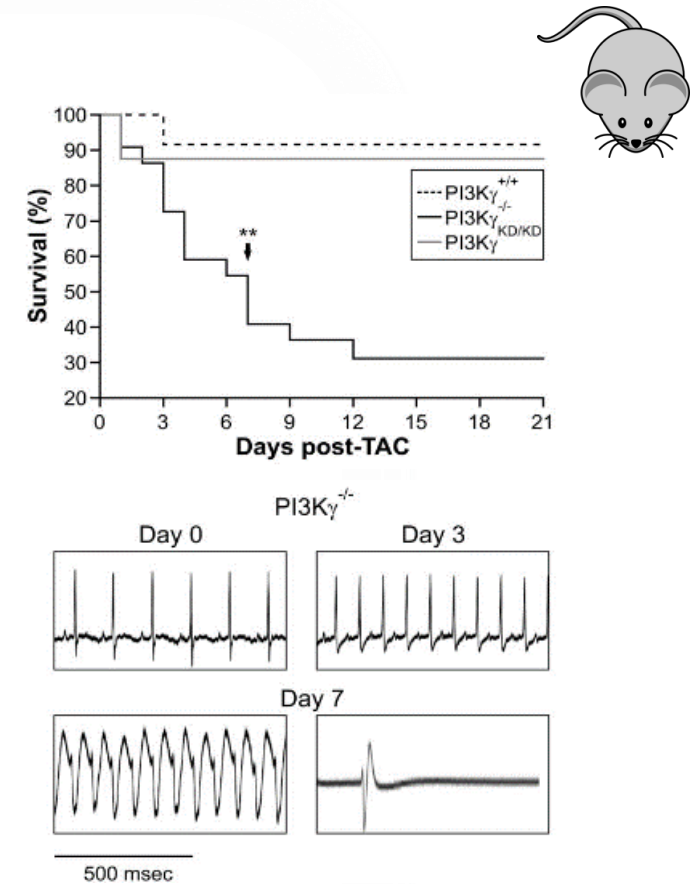


Ghigo et al., *Circulation* 2012

# $\beta_2$ -AR-Induced Lethal Arrhythmias in PI3K $\gamma$ KO but not PI3K $\gamma$ KD Mice



Ghigo & Mika, *J Mol Cell Cardiol.* 2019



Ghigo et al., *Circulation* 2012

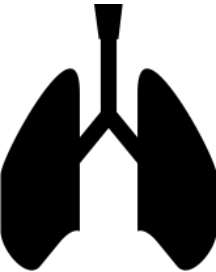
# What's Bad for the Heart Might be Good for the Lungs!

cAMP is:

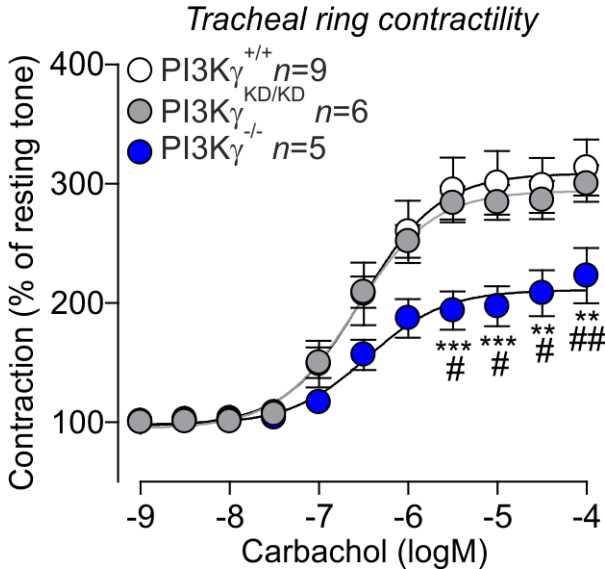


**BAD**  
for the Heart  
*Heart failure:*  
*β-blockers*

BUT

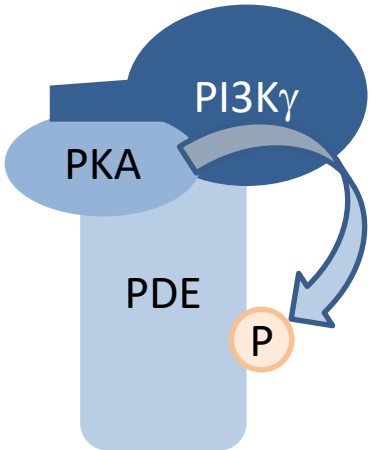


**GOOD**  
for the LUNGS  
*Asthma:*  
*Albuterol*



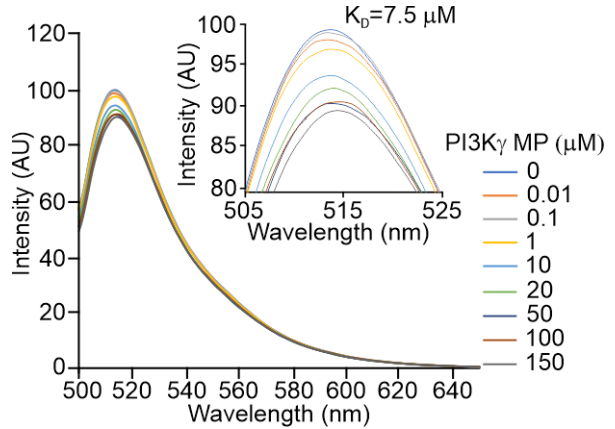
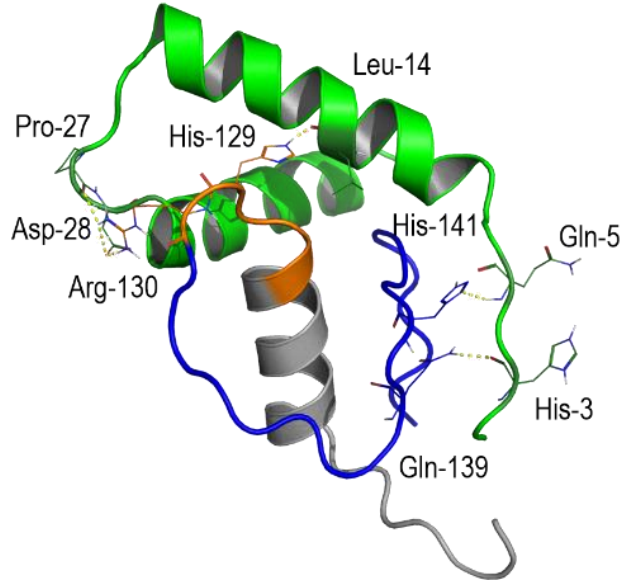
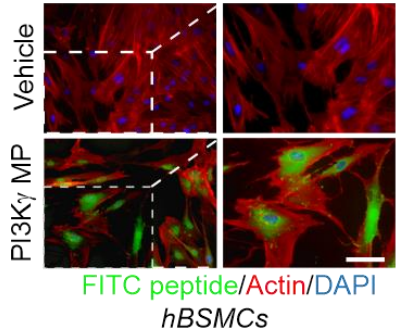
# Targeting the AKAP function of PI3K $\gamma$ with a Cell-Penetrating Mimetic Peptide

PI3K $\gamma$  MP



## PI3K $\gamma$ MP internalization in Human Bronchial Smooth Muscle Cells

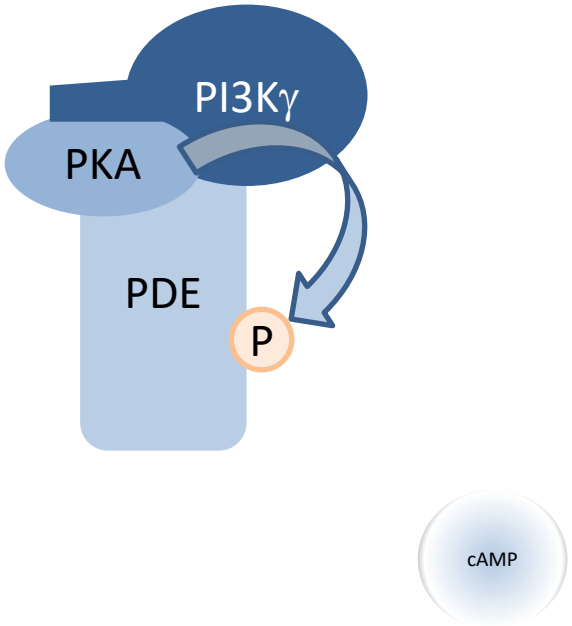
PI3K $\gamma$  mimetic peptide (MP)



Hirsch et al., Science 2000  
Crackower et al., Cell 2002  
Patrucco et al., Cell 2004  
Perino et al. Mol Cell 2011  
Ghigo et al., Circulation 2012  
Ghigo et al. Circ Res 2017  
Lupieri et al. J Cell Sci 2020

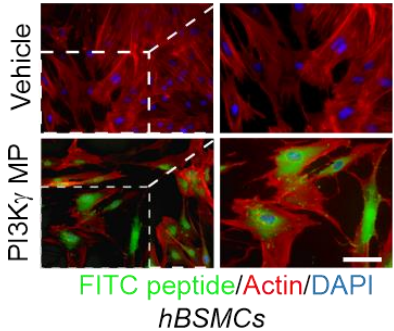
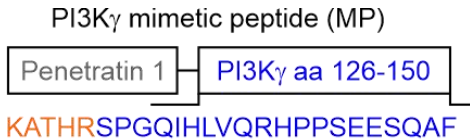
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PI3K $\gamma$  MP

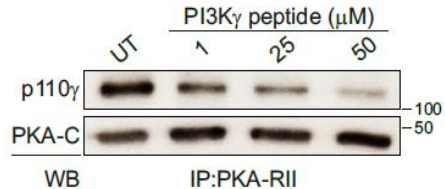


Hirsch et al., Science 2000  
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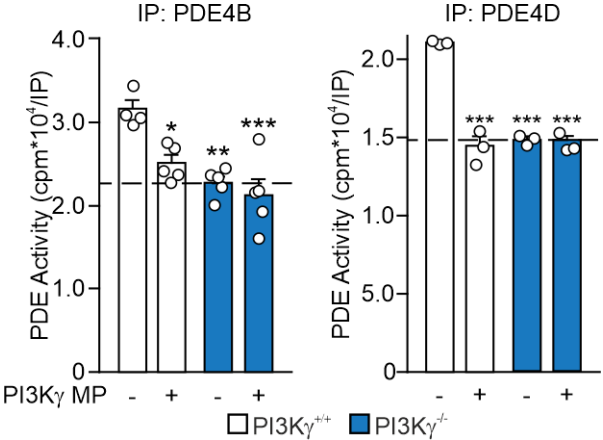
### PI3K $\gamma$ MP internalization in Human Bronchial Smooth Muscle Cells



### Disruption of PKA/PI3K $\gamma$ binding by the PI3K $\gamma$ MP

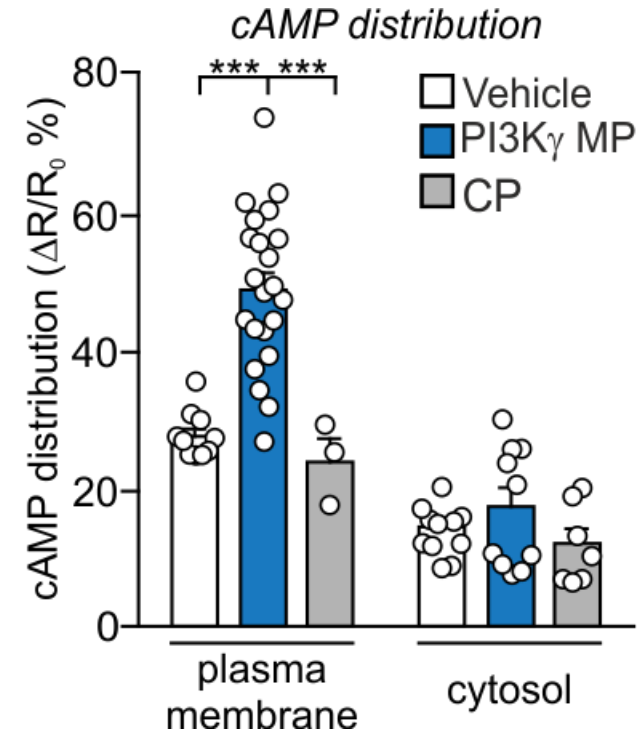
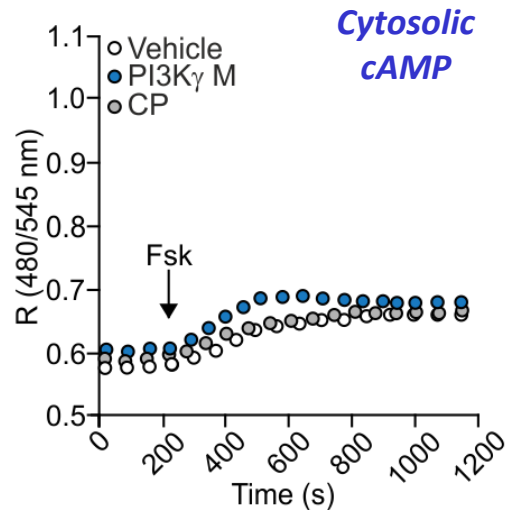
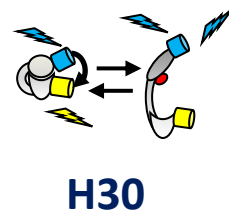
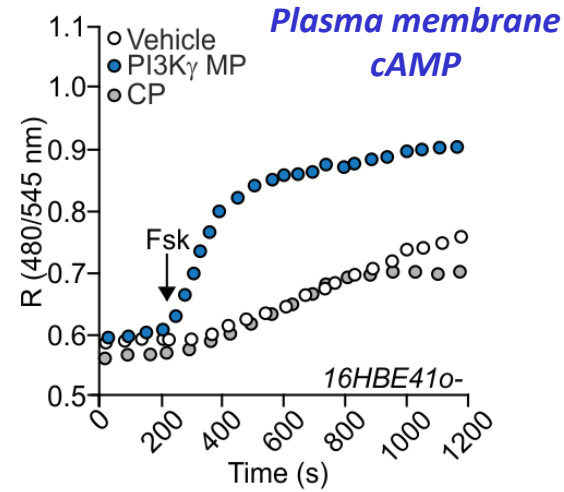
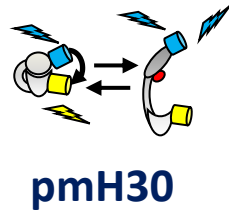


### PDE4B and PDE4D inhibition by the PI3K $\gamma$ MP

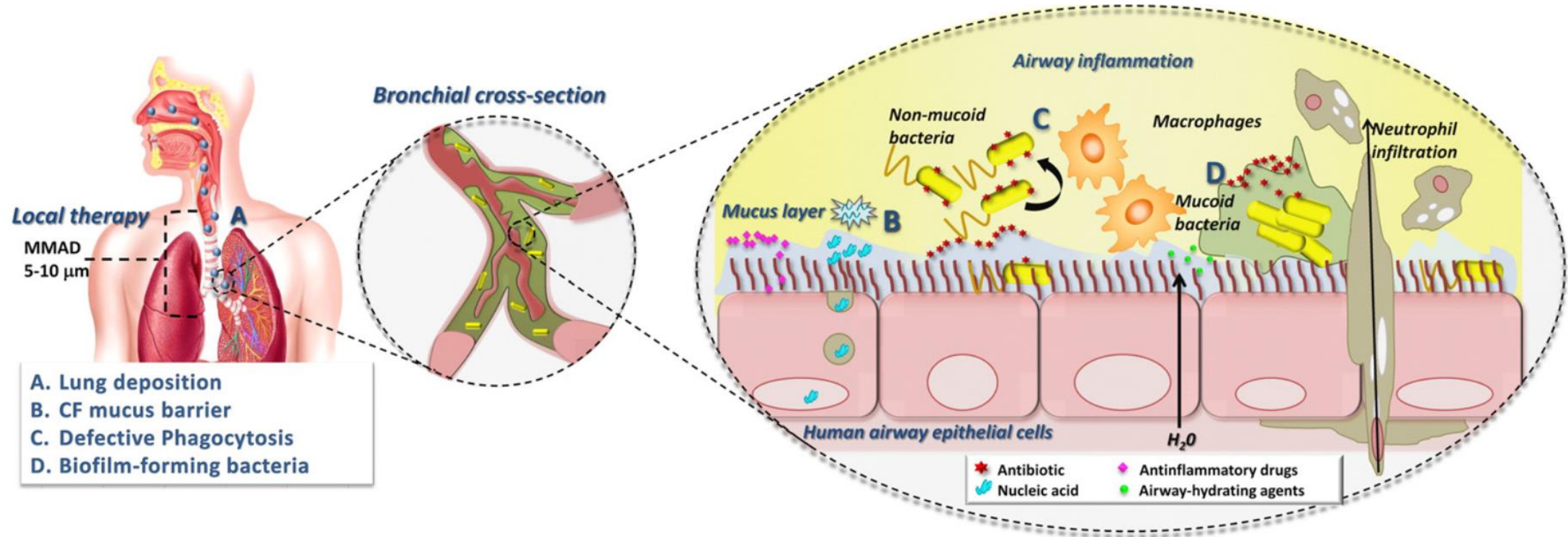




# PI3K $\gamma$ MP Triggers a Compartmentalized cAMP Elevation in Lung Cells



# Challenges in the Development of Inhaled Treatments for Airway Diseases

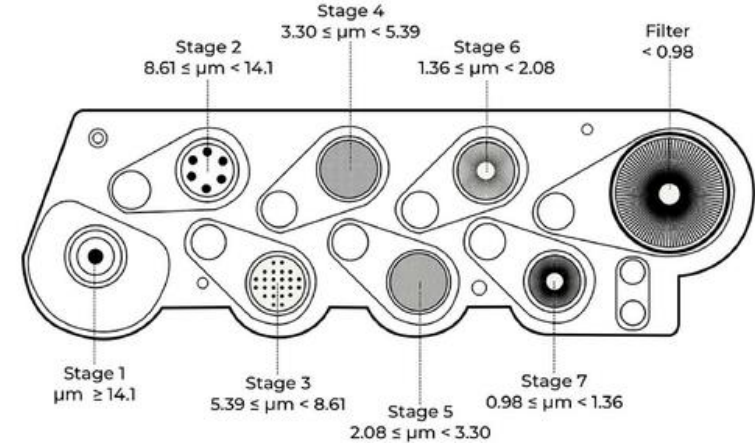
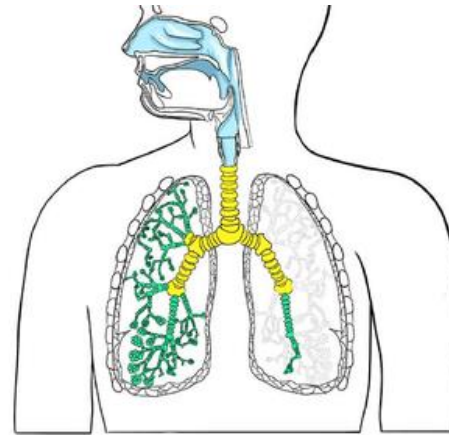


# PI3Ky MP is Suitable for Inhaled Therapy

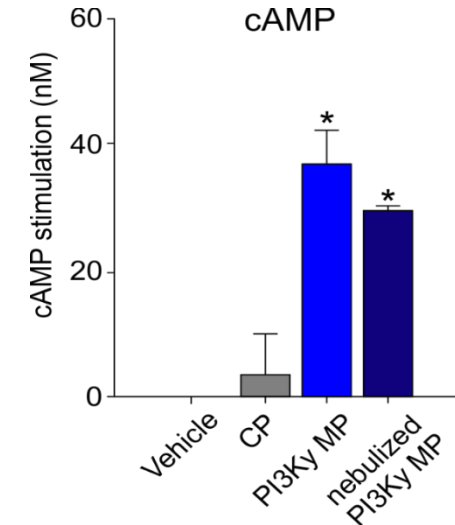
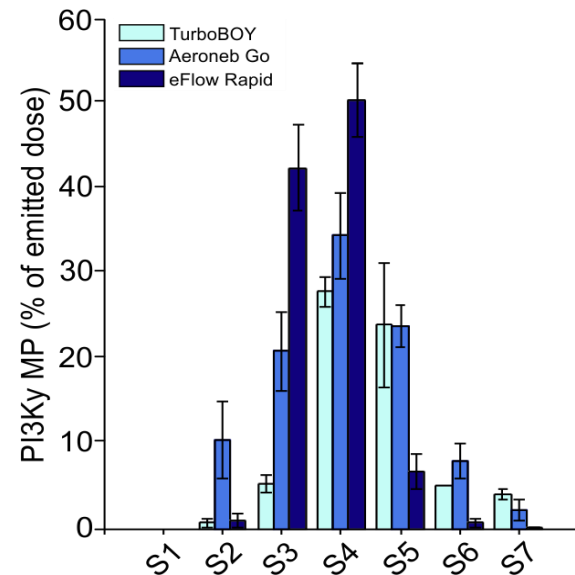
## Next generation impactor (NGI)



- Upper airways (naso/oropharynx and larynx)**  
Approx. IP, Stage 1,2,3 (5  $\mu$ m)
- Central airways (Trachea, primary and secondary bronchi)**  
Approx. Stage 4,5 (2-5  $\mu$ m)
- Peripheral airways (tertiary bronchi, terminal bronchioles and alveoli)**  
Approx. Stage 6,7 (0,5-2  $\mu$ m)

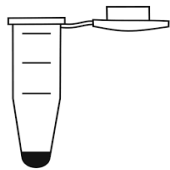


|  | NGI Cup | Particles (micron) |
|--|---------|--------------------|
|  | S1      | 14.10              |
|  | S2      | 8.61               |
|  | S3      | 5.39               |
|  | S4      | 3.30               |
|  | S5      | 2.08               |
|  | S6      | 1.36               |
|  | S7      | 0.98               |



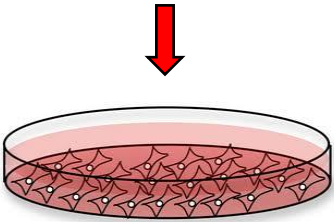
# PI3K $\gamma$ MP is Suitable for Inhaled Delivery

### 1. PI3K $\gamma$ MP + HNE

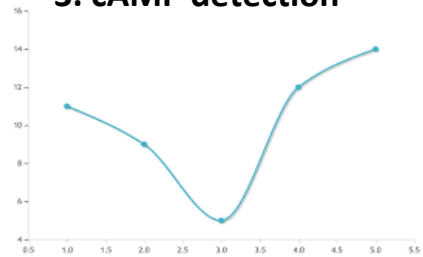


directly on  
16HBE cells

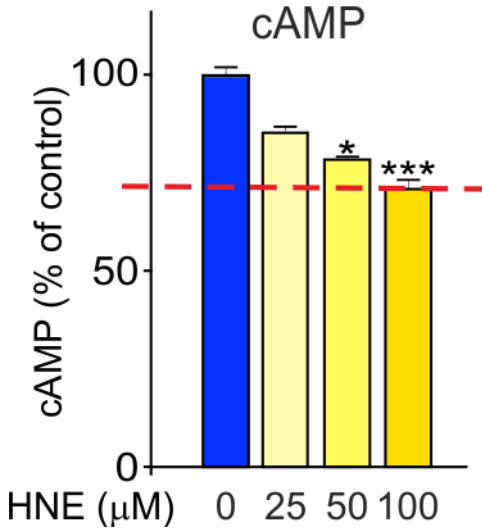
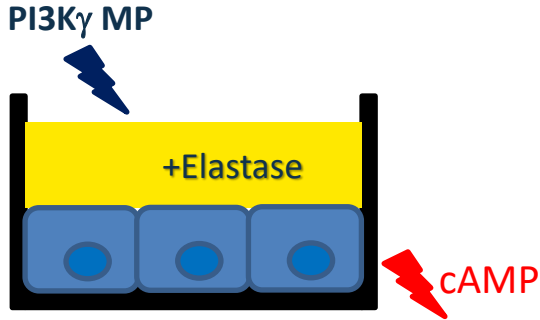
### 2. Cell treatment



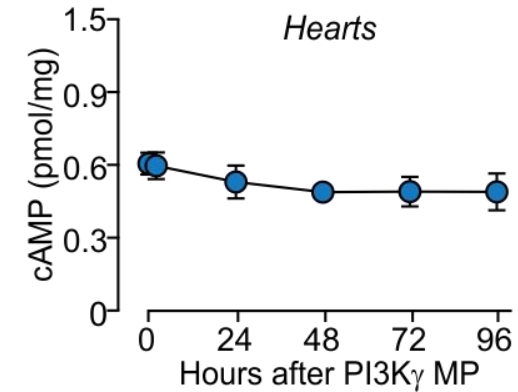
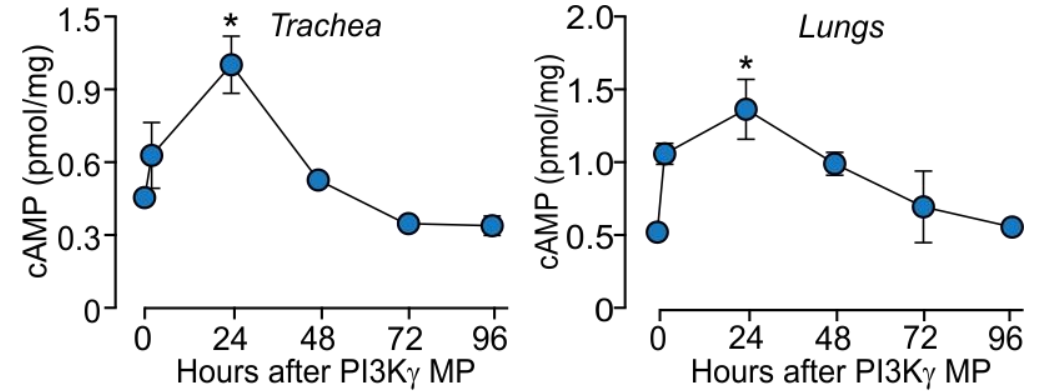
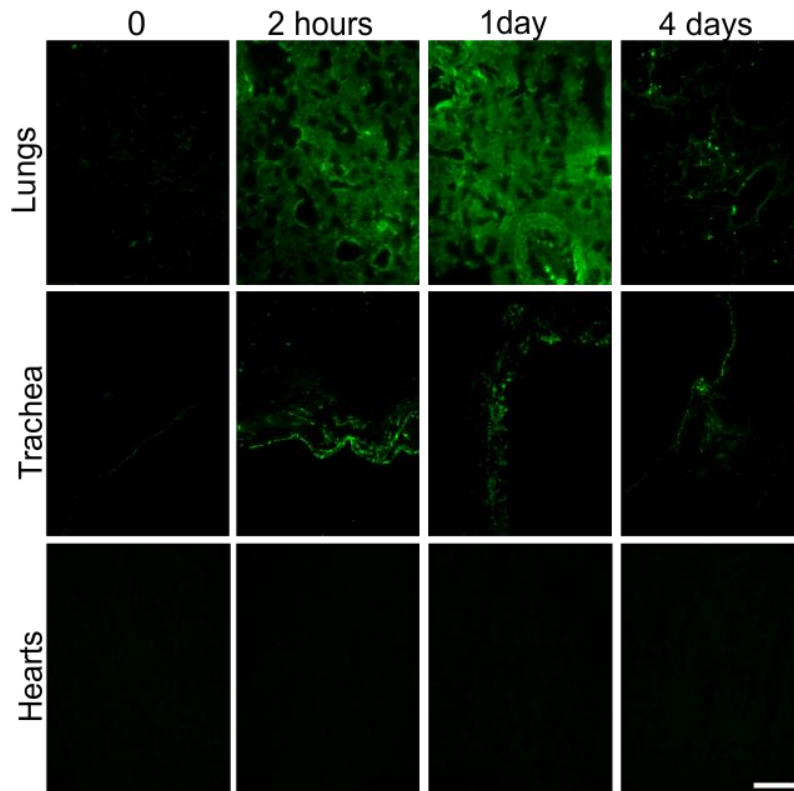
### 3. cAMP detection



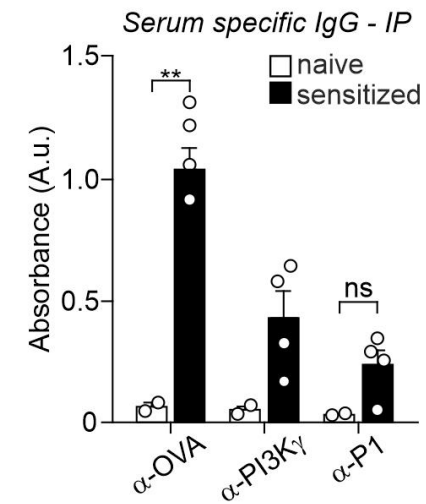
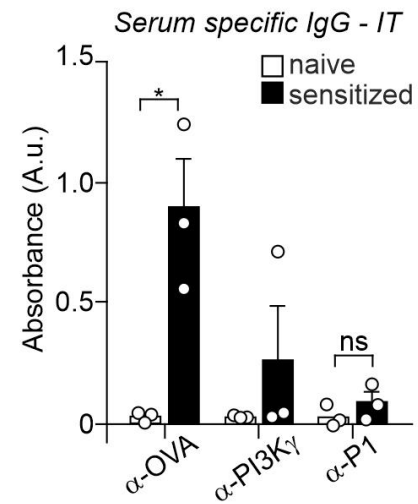
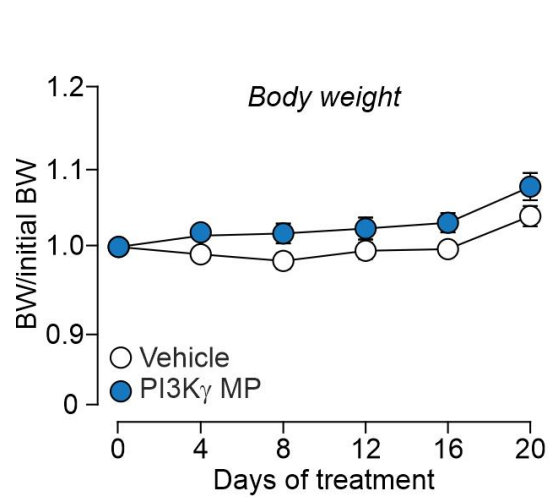
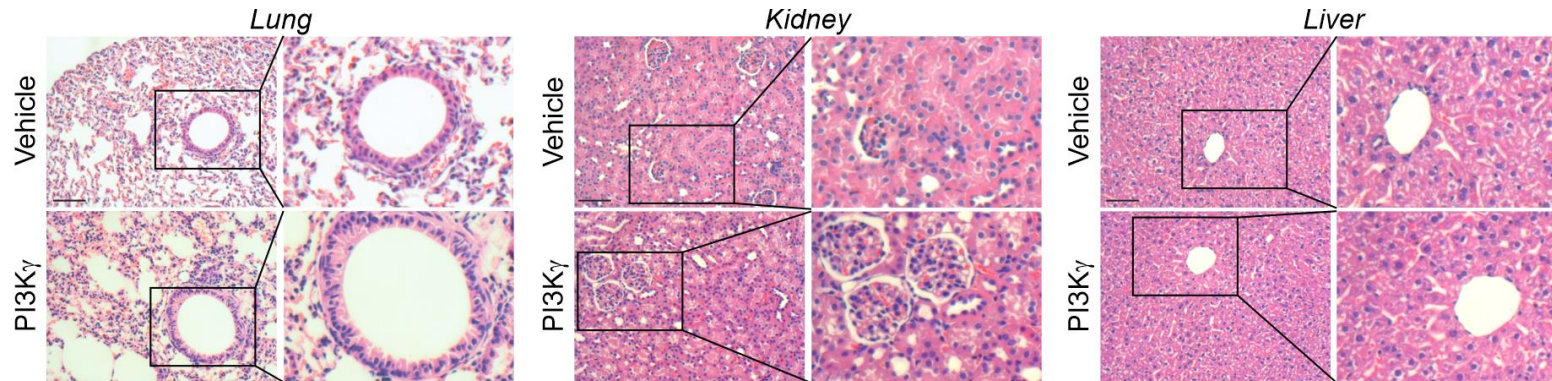
*Resistance to Proteases  
(Human neutrophil elastase - HNE)*



# Inhaled PI3K $\gamma$ MP Promotes Long-lasting cAMP Elevation in the Lungs



# PI3K $\gamma$ MP has a Good Tolerability Profile



# Therapeutic Effects of PI3K $\gamma$ MP



Smooth Muscle Cells

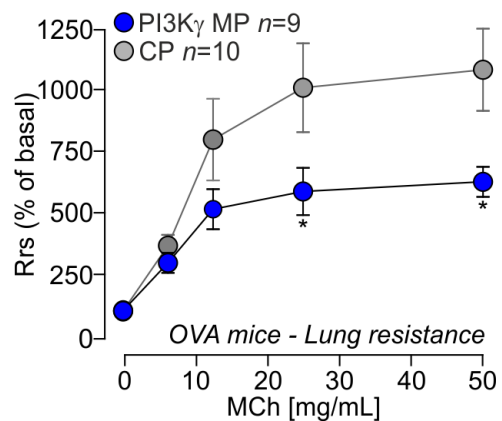


Inflammatory Cells

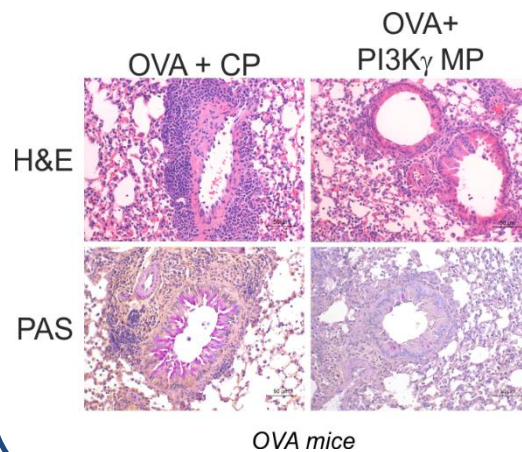


Epithelial Cells

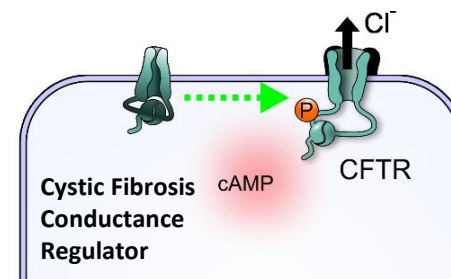
## Bronchodilation



## Anti-inflammatory effect



## CFTR modulator



# Therapeutic Effects of PI3K $\gamma$ MP



Smooth Muscle Cells

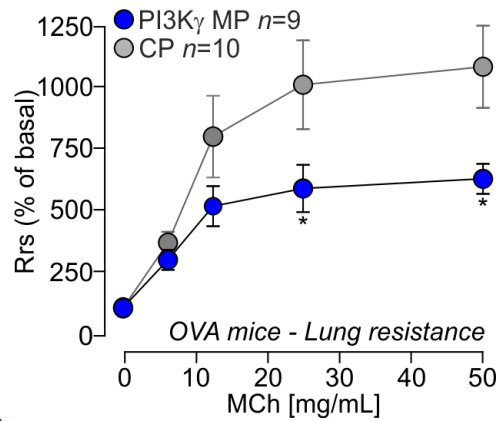


Inflammatory Cells

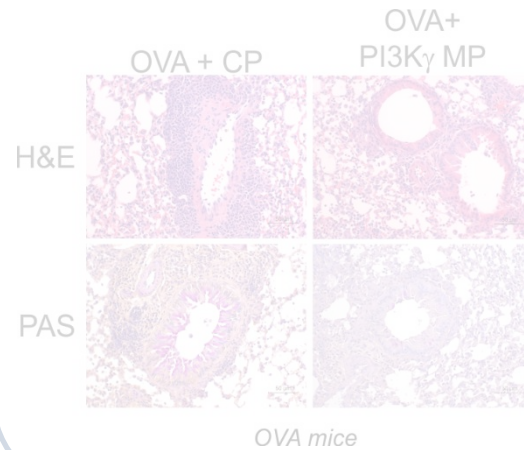


Epithelial Cells

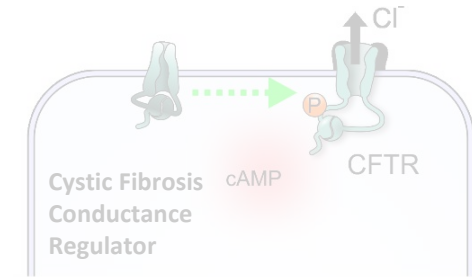
## Bronchodilation



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# Therapeutic Effects of PI3K $\gamma$ MP



Smooth Muscle Cells

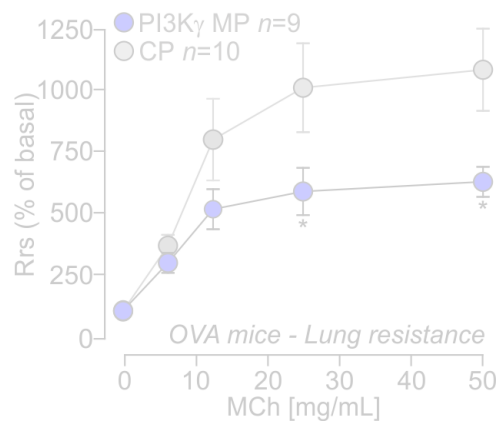


Inflammatory Cells

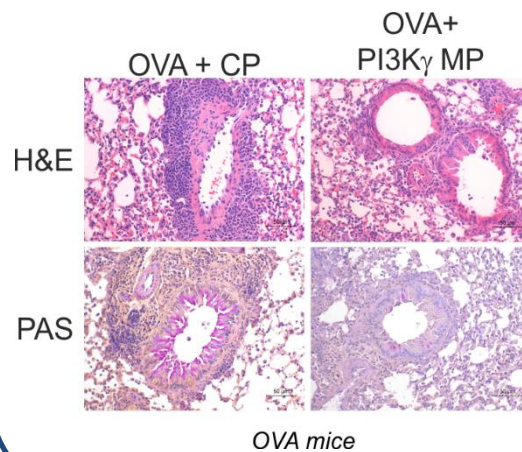


Epithelial Cells

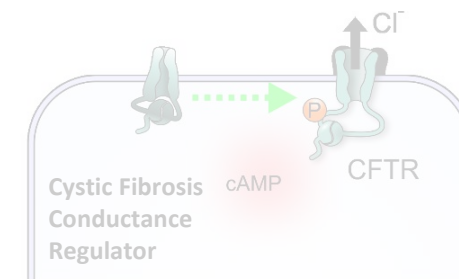
## Bronchodilation



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## CFTR modulator



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Smooth Muscle Cells

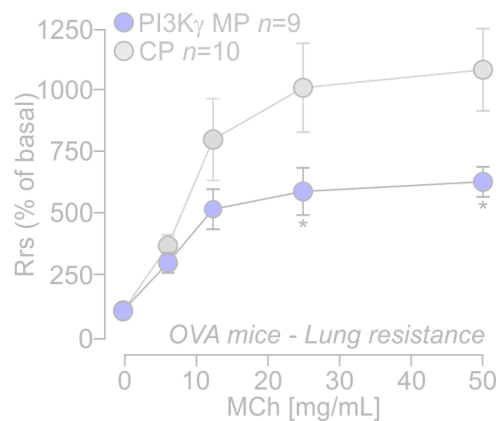


Inflammatory Cells

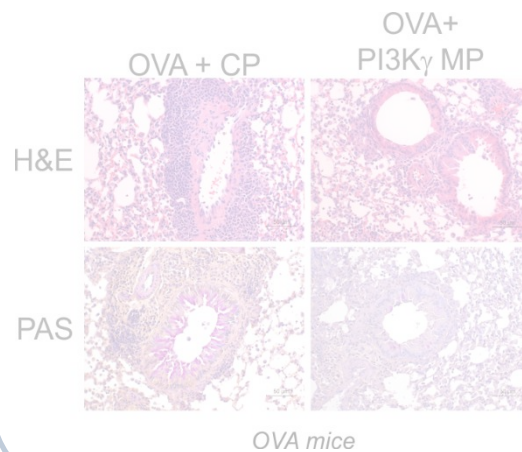


Epithelial Cells

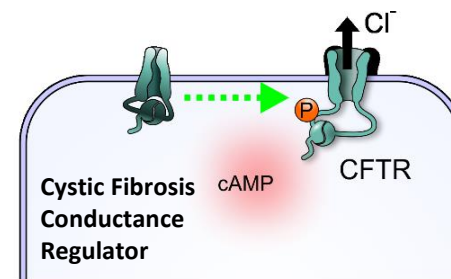
## Bronchodilation



## Anti-inflammatory effect



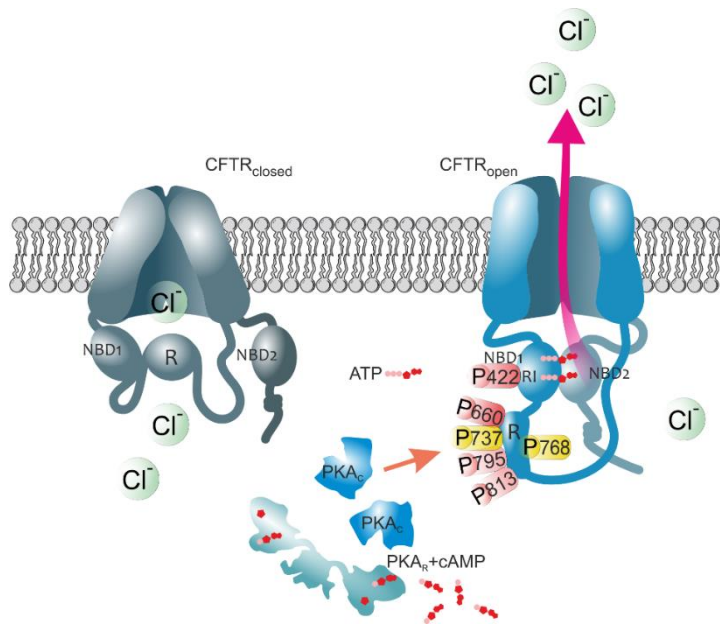
## CFTR modulator



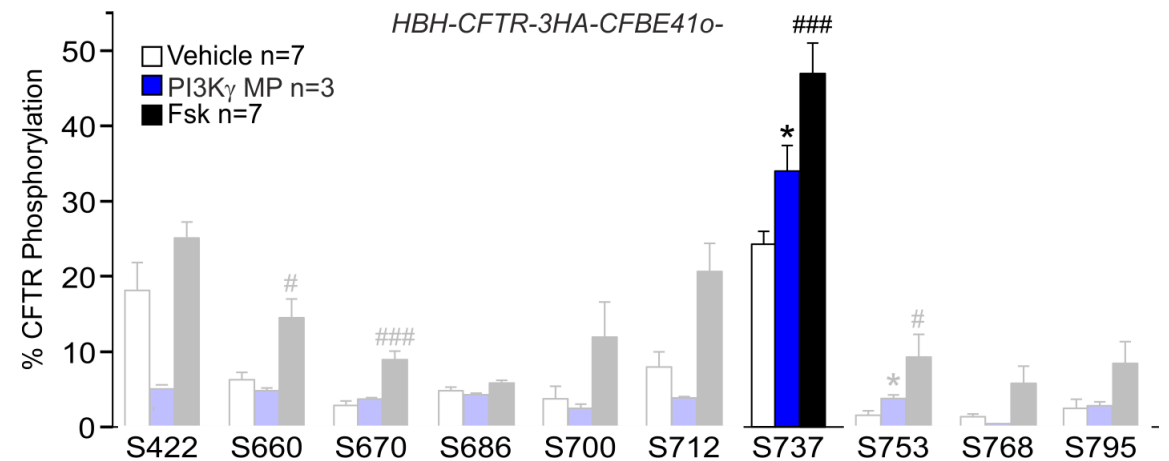
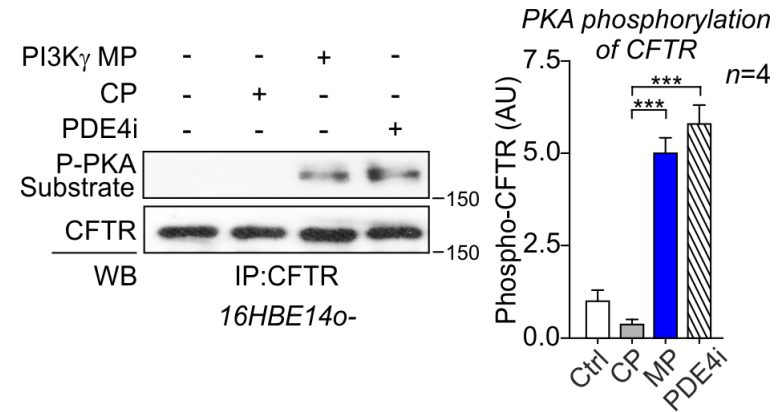
# The PI3K $\gamma$ MP promotes cAMP-mediated phosphorylation of the CFTR



Epithelial Cells



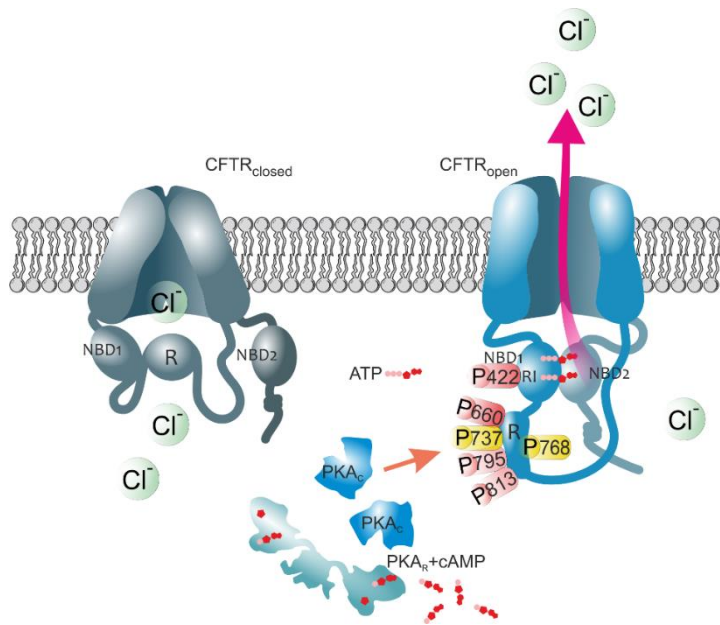
Della Sala A (and Ghigo) Front Physiol 2021



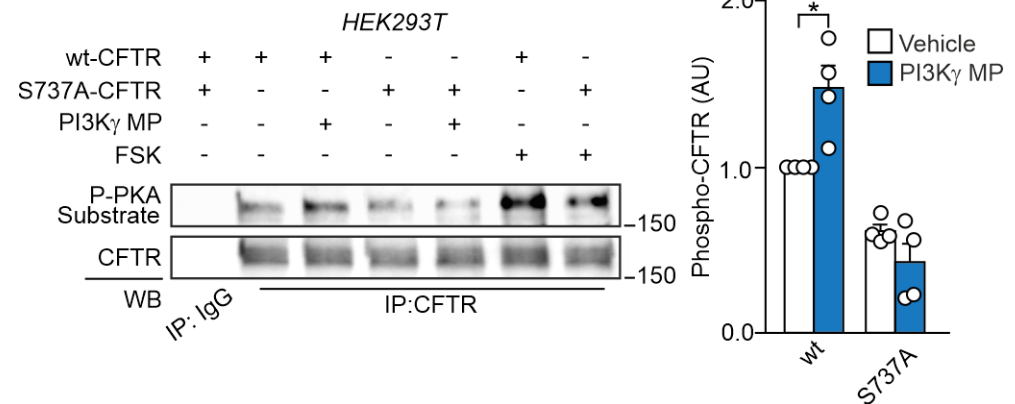
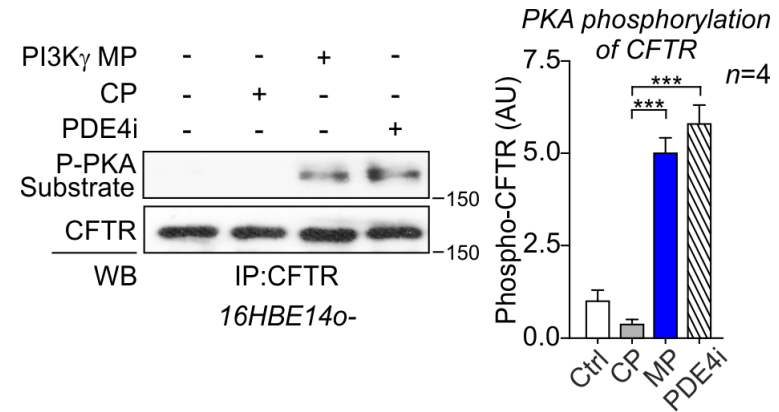
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Epithelial Cells



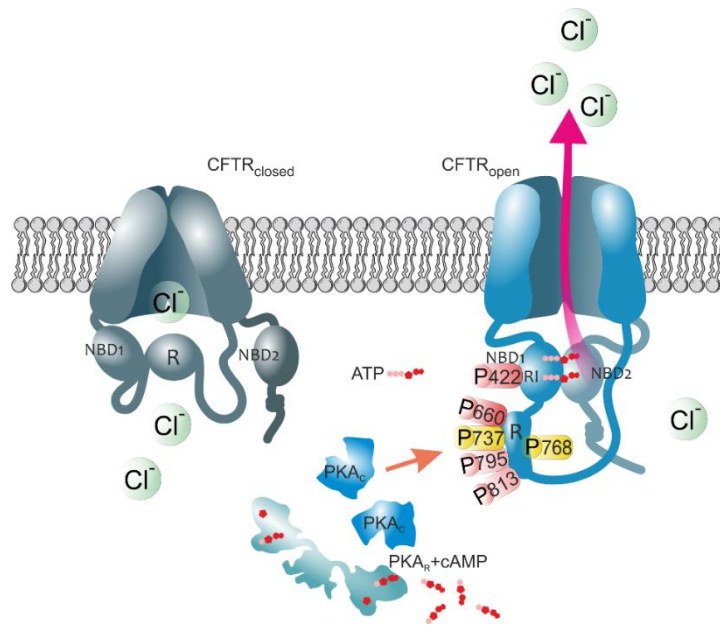
Della Sala A (and Ghigo) Front Physiol 2021



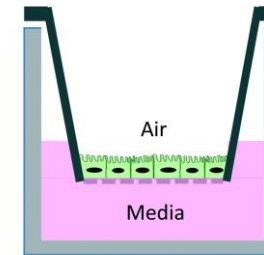
# The PI3K $\gamma$ MP Triggers CFTR Opening



Epithelial Cells

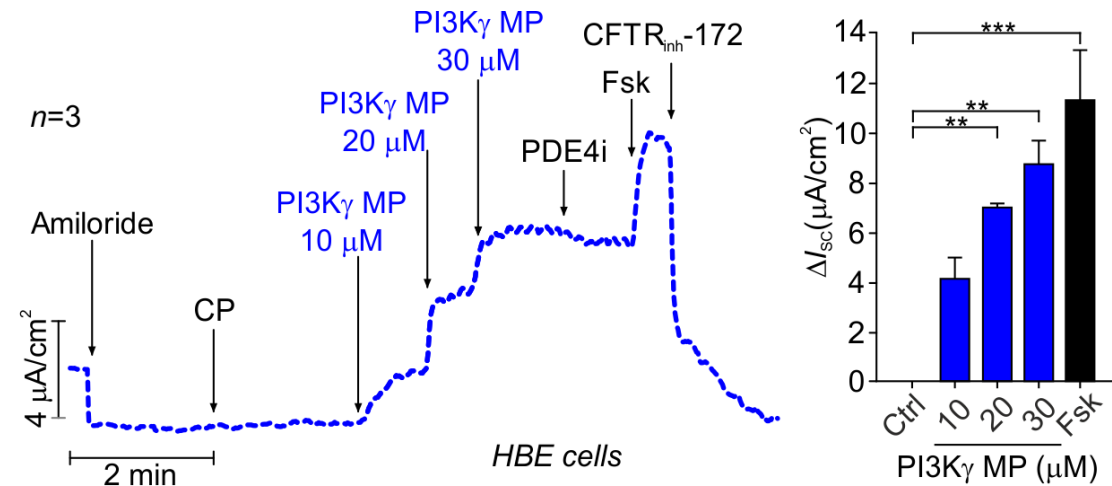


Della Sala A (and Ghigo) Front Physiol 2021



Air-Liquid Interface

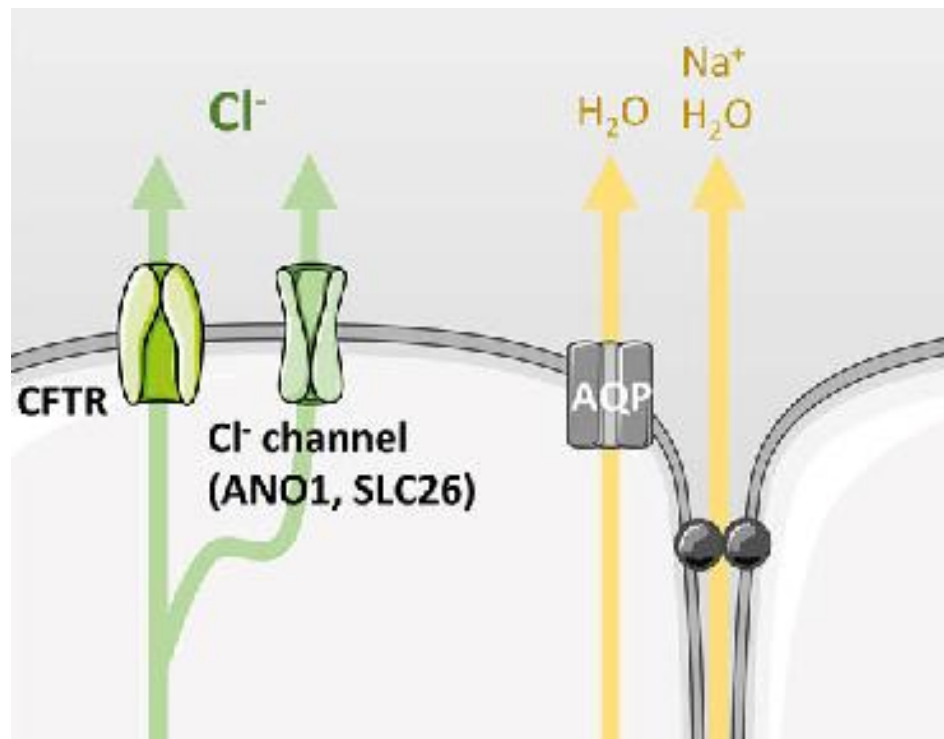
*Short circuit current measurements in human primary bronchial epithelial cells*



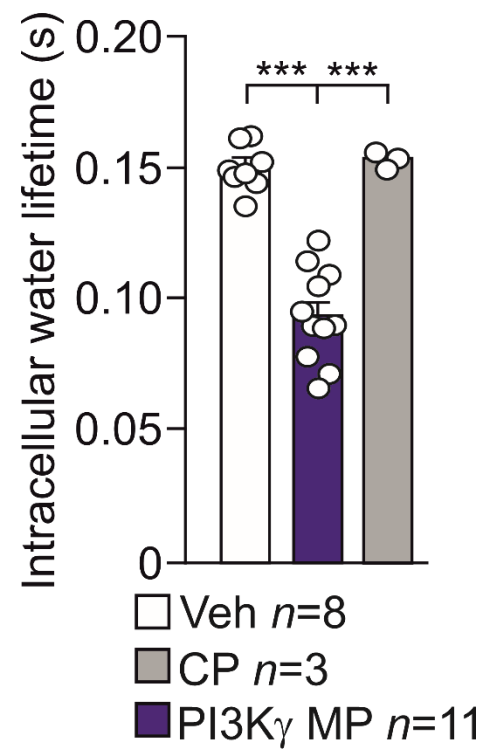
# The PI3K $\gamma$ MP Induces Water Secretion from Epithelial Cells



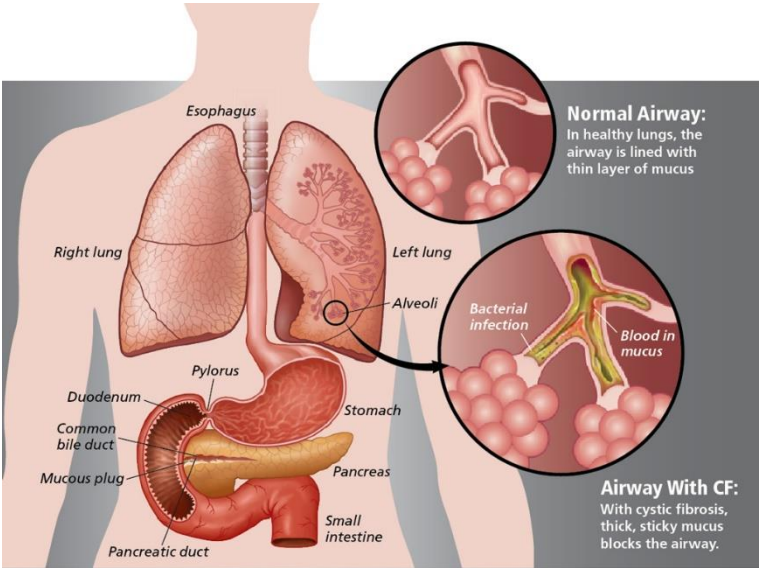
Mucus Hydration



Water residence time ( $\tau_{in}$ ) determined by <sup>1</sup>H NMR relaxometry



# CFTR Dysfunction is the Basic Defect in Cystic Fibrosis

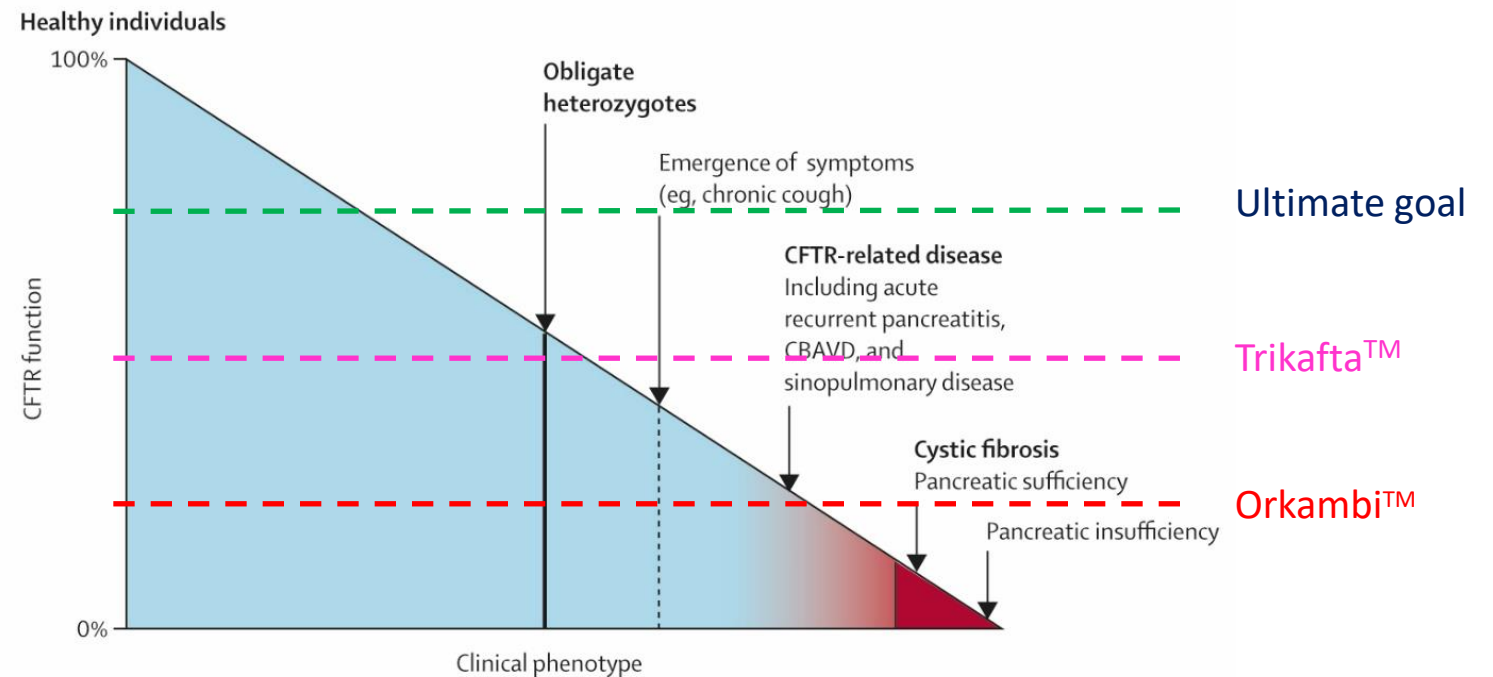
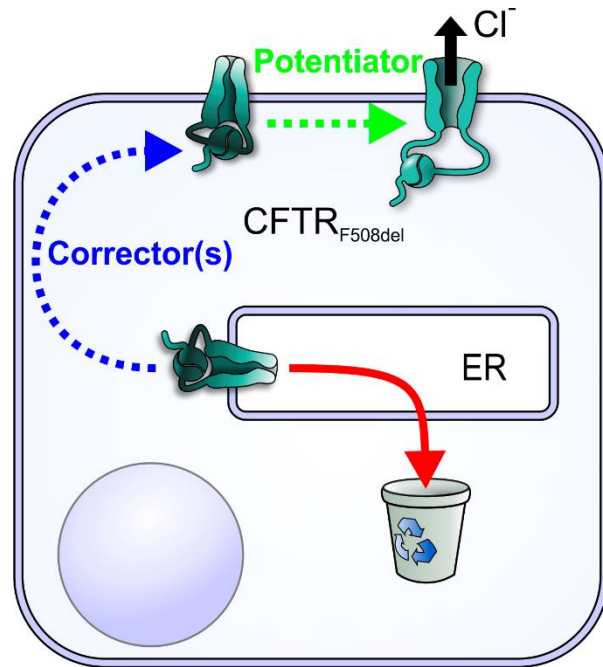


- Cystic Fibrosis is the **most common among rare genetic diseases**, affecting more than 80000 patients worldwide
- It is caused by mutations in the *CFTR* gene, encoding for a **cAMP-regulated chloride channel**
- It is a multiorgan syndrome, with respiratory failure being the major cause of death
- **Median predictive survival: 44 years**
- **No effective treatment available**

90% of patients:  
**F508del**

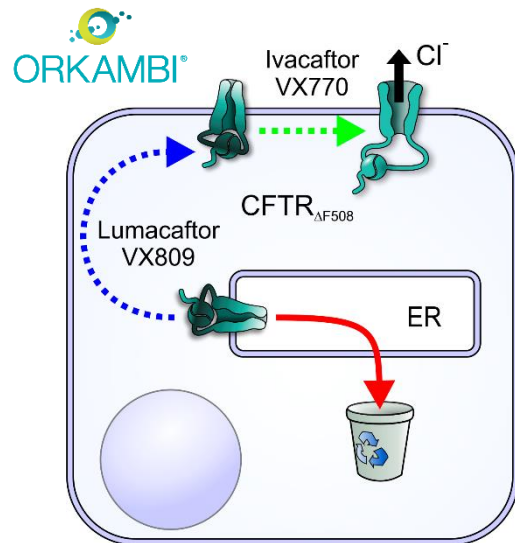
|                        | Class of mutation                         |                                                   |                                                  |                                                  |                                              |                                    |
|------------------------|-------------------------------------------|---------------------------------------------------|--------------------------------------------------|--------------------------------------------------|----------------------------------------------|------------------------------------|
|                        | Normal                                    | I                                                 | II                                               | III                                              | IV                                           | V                                  |
| Molecular defect       | No synthesis                              | Block in processing                               | Block in regulation                              | Reduced conductance                              | Reduced synthesis                            | Reduced half-life                  |
| Functional abnormality | Protein is not synthesized                | Folding defect                                    | Channel opening defect                           | Ion transport defect                             | Decreased protein synthesis                  | Decreased half-life of the protein |
| Main mutations         | Gly542X<br>Trp128X<br>Arg553X<br>621+1G→T | Phe508del<br>Asn1303Lys<br>Ile507del<br>Arg560Thr | Gly551Asp<br>Gly178Arg<br>Gly551Ser<br>Ser549Asn | Arg117His<br>Arg347Pro<br>Arg117Cys<br>Arg334Trp | 3849+10kbC→T<br>2789+5G→A<br>3120+1G→A<br>5T | 4326delTC<br>Gln1412X<br>4279insA  |

# Approved CFTR modulators only Partially Rescue F508del-CFTR Function



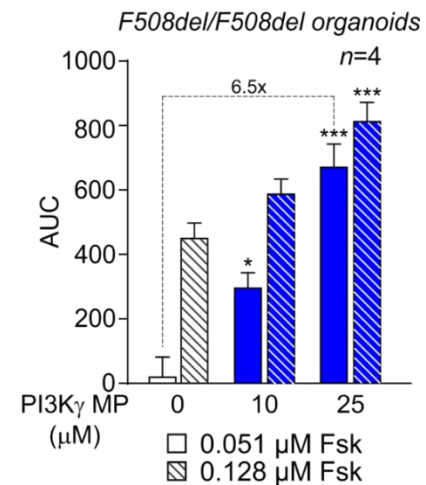
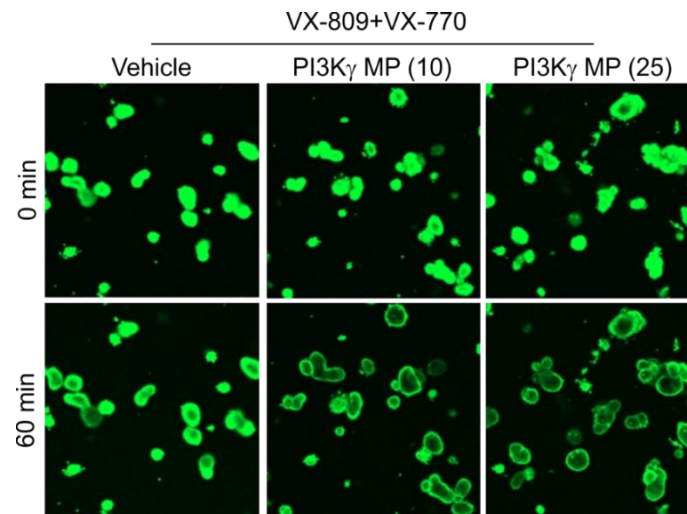
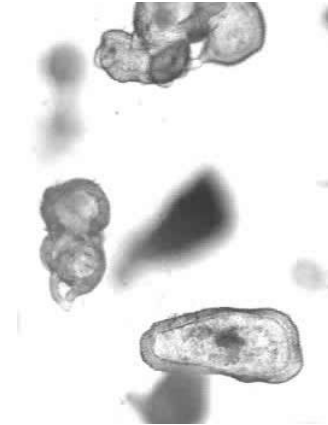
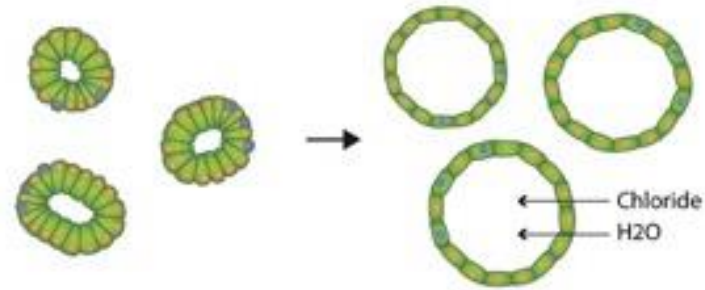


# The PI3K $\gamma$ MP Potentiates the Effect of CFTR Modulators by 6 folds in F508del Organoids

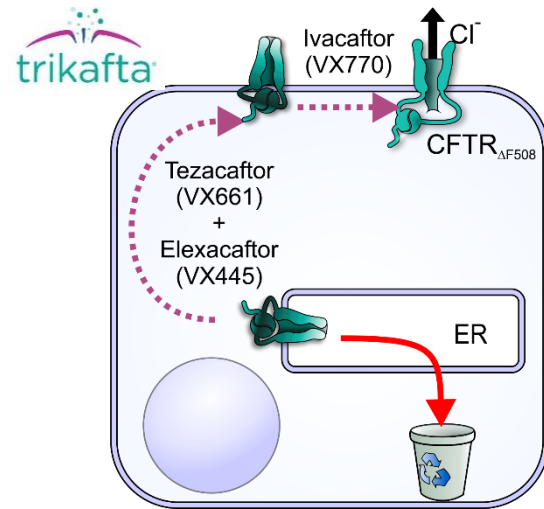


Approved by FDA in 2015 despite limited efficacy (<4% lung function improvement)

Forskolin-induced swelling assay in patient-derived organoids

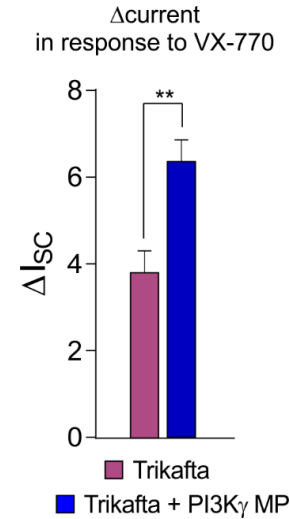
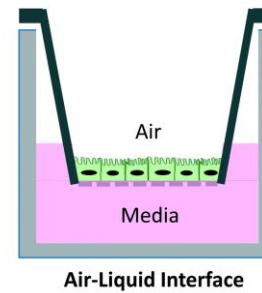


# The PI3K $\gamma$ MP Doubles the Effect of the Recently Approved Combination Trikafta™

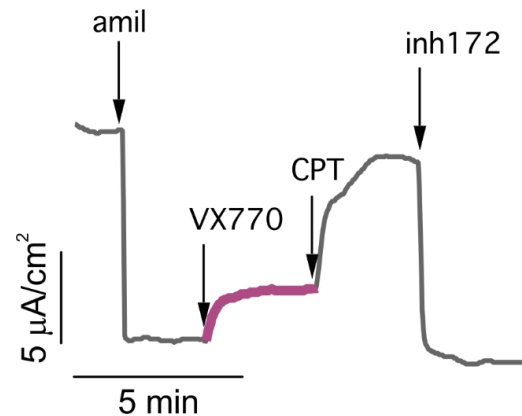


Approved by FDA in 2019  
(by EMA in 2020)

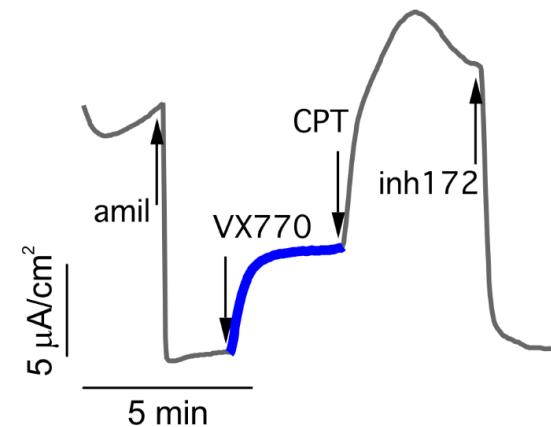
Short circuit current measurements in patient-derived bronchial epithelial cells (F508del/F508del)



Trikafta™ alone  
VX-445+VX-661 (24h)

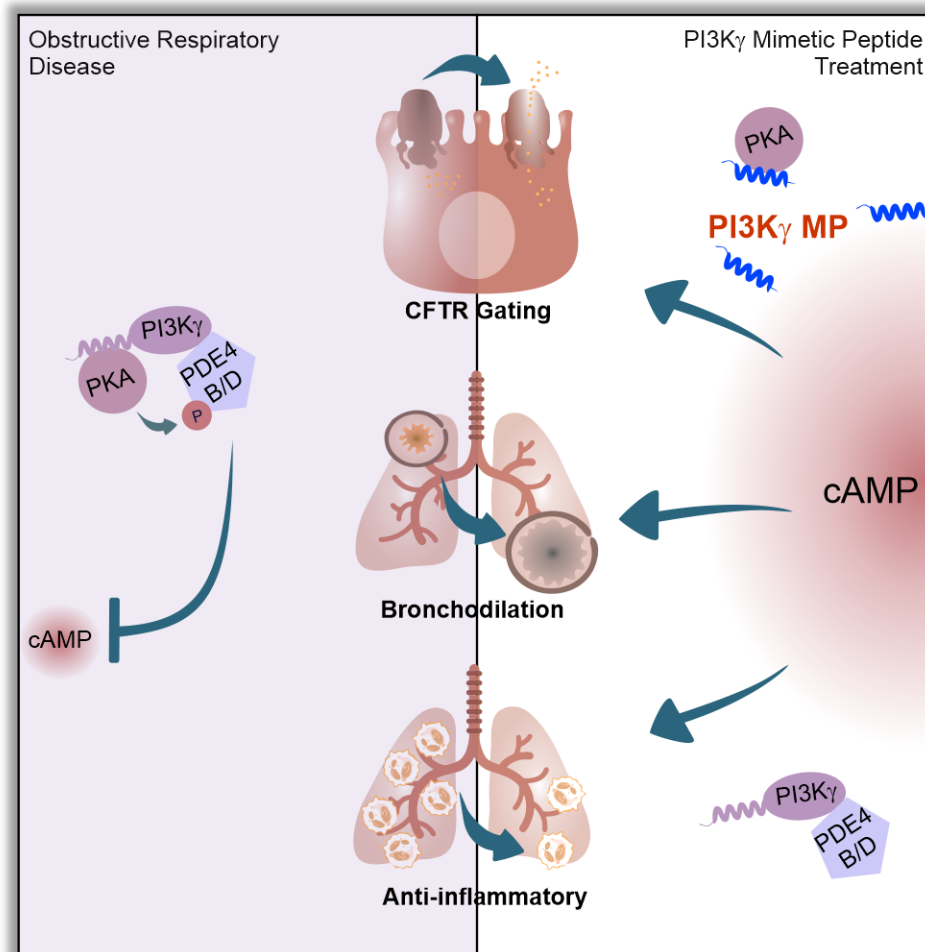


Trikafta™ + PI3K $\gamma$  MP  
VX-445+VX-661+peptide (24h)



N=2 independent patients

# Conclusion



- PI3K $\gamma$  MP inhibits a specific pool of subcortical PDE4B and D increasing cAMP in a compartments that contain  $\beta$ 2-ARs
- Inhaled PI3K $\gamma$  MP promotes long-lasting cAMP elevation in the lungs, triggering airway smooth muscle relaxation and reduced neutrophil infiltration
- In epithelial cells, PI3K $\gamma$  MP elevates cAMP in the vicinity of CFTR triggering PKA-mediated phosphorylation of the activating serine 737
- PI3K $\gamma$  MP potentiates the effects of existing CFTR modulators in rescuing F508del-CFTR function

### *Future directions:*

- Does PI3K $\gamma$  MP directly rescue the function of cAMP-responsive CFTR mutants, like class III-IV R117H, A455E, R334W, T338I, G551D, S549R?

## KIT2014 as Orphan Drug

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**KIT2014** (Pat. Appl. WO 2016/103176)



Patent Approved in EU  
& USA

- Orphan Drug Designation (Feb. 2017) by European Medicine Agency (EMA)

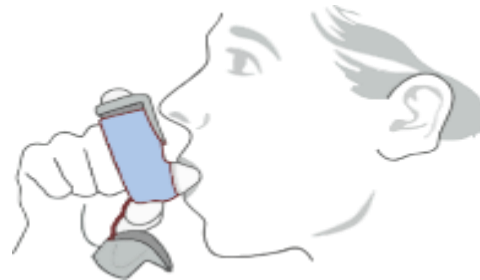


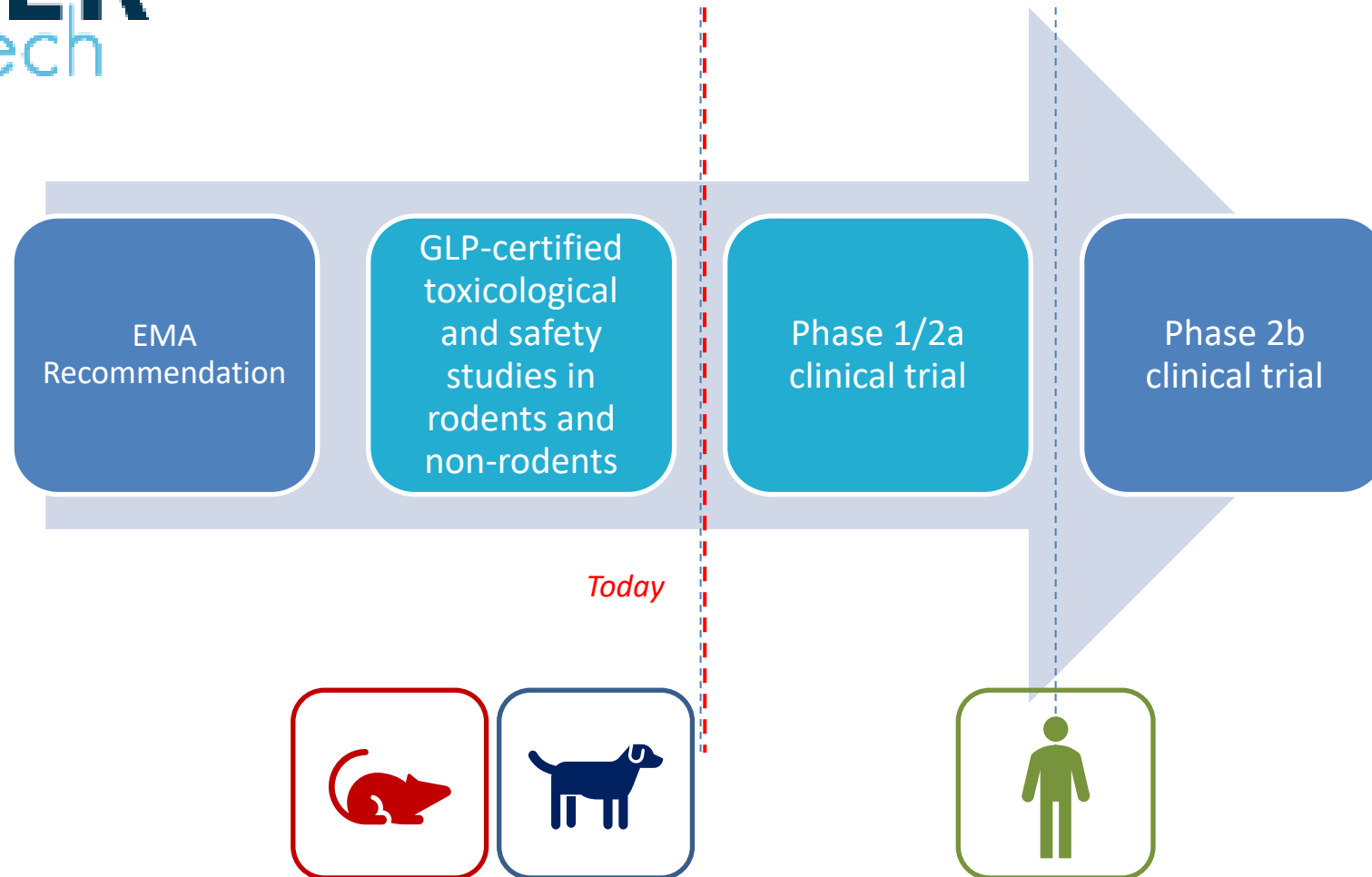
Orphan Drug  
Designation

- “Scientific Advice” obtained from EMA for preclinical development (Oct. 2018)



Scientific Advice  
from EMA





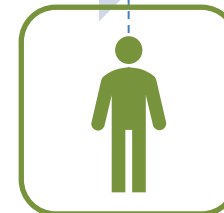
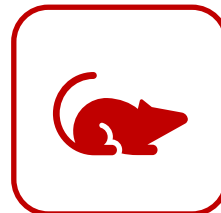
EMA  
Recommendation

GLP-certified  
toxicological  
and safety  
studies in  
rodents and  
non-rodents

Phase 1/2a  
clinical trial

Phase 2b  
clinical trial

*Today*



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fondazione per la ricerca  
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