

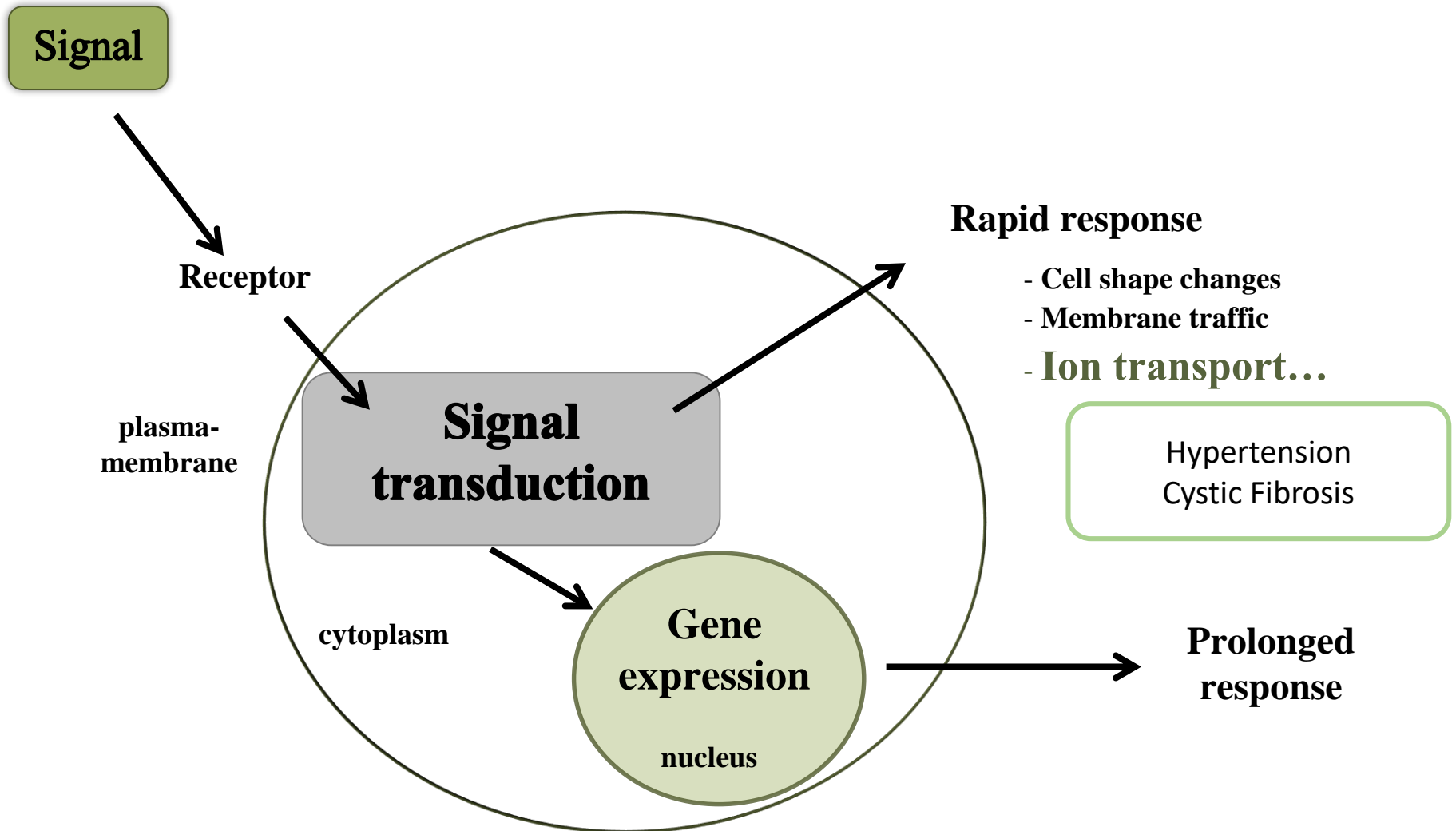
**Ciências
ULisboa**



A SYK/SHC1 pathway regulates the amount of CFTR in the plasma membrane

**Cláudia Almeida Loureiro, Francisco R Pinto,
Patrícia Barros, Paulo Matos, Peter Jordan**

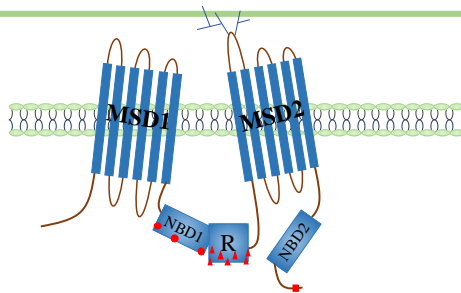
Extracellular signals and cellular response



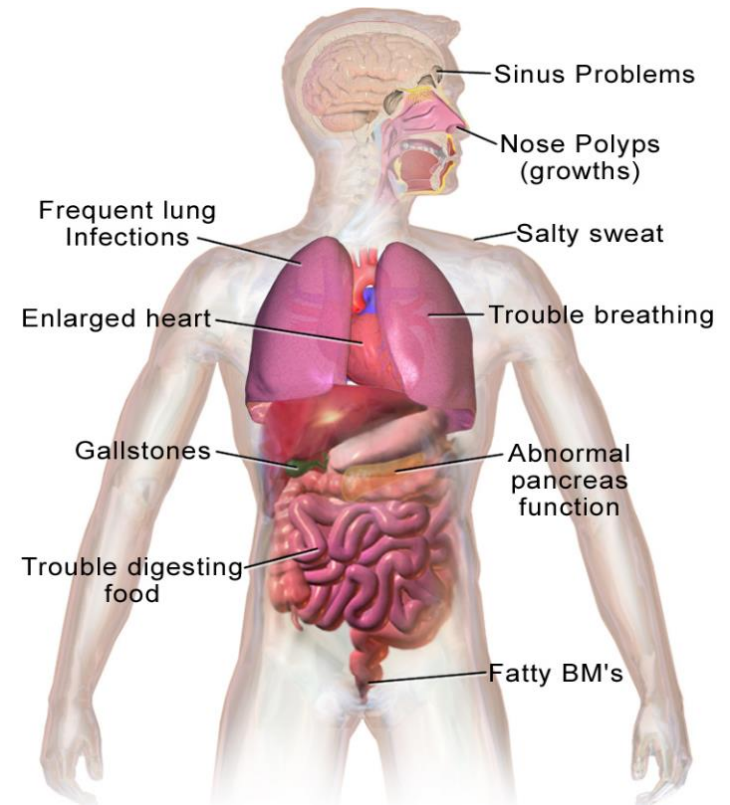
Cystic fibrosis

- The most frequent genetic disease in the Caucasian population
- Affect more than 70 000 individuals worldwide
- Production of abnormally thick mucus
 - Recurrent respiratory infections
 - Pancreatic insufficiency

Cystic fibrosis transmembrane conductance regulator (CFTR)

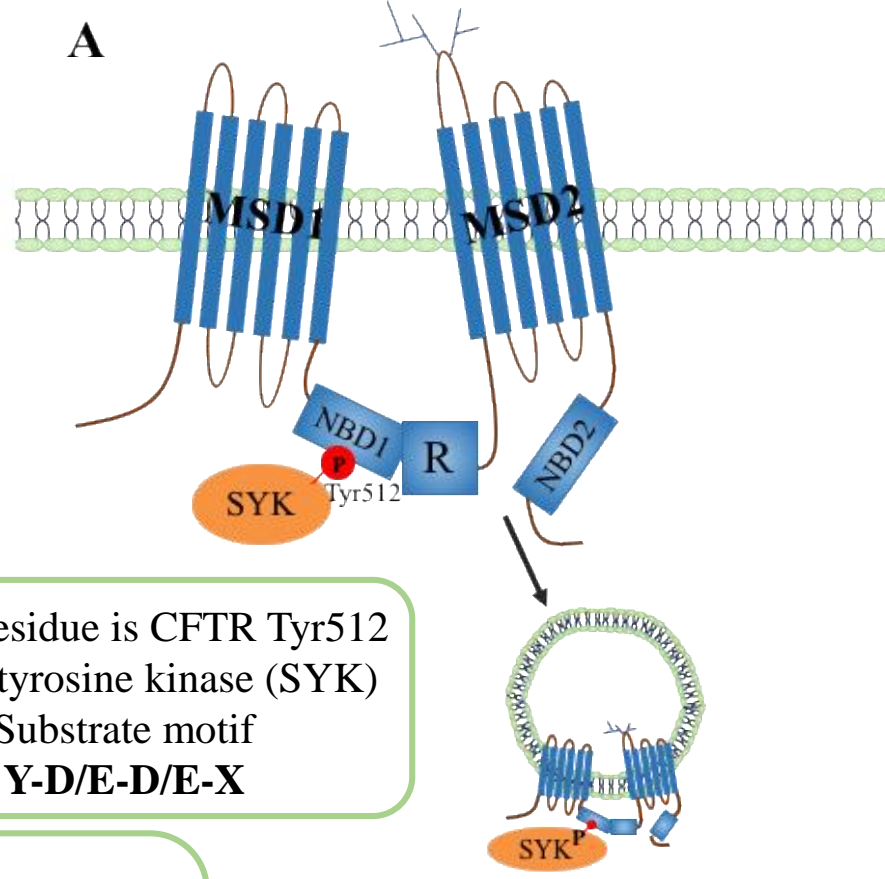
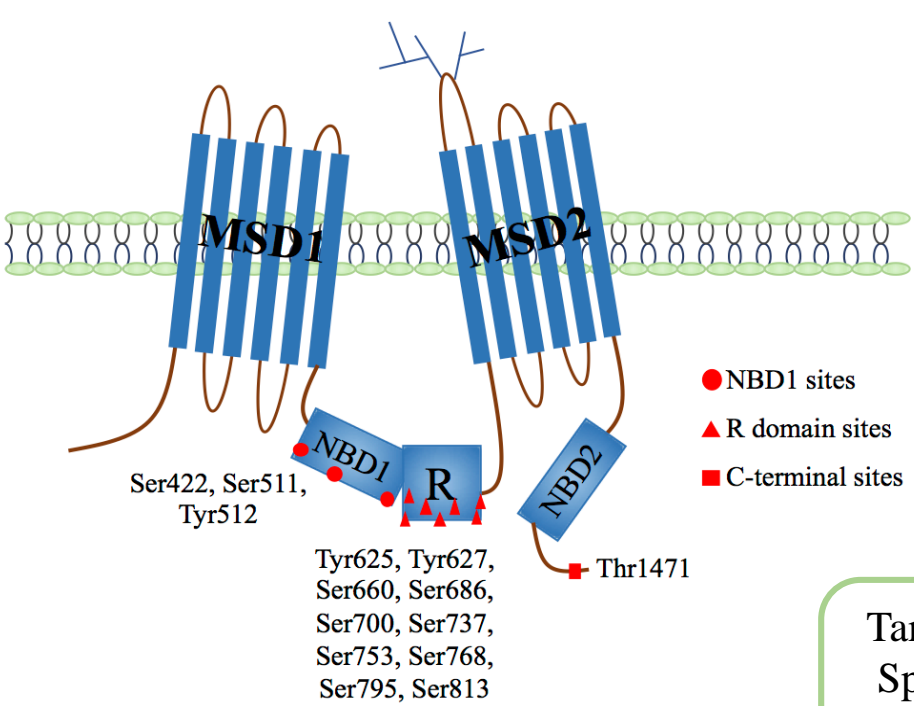


Over **2000** mutations causing cystic fibrosis



Multisystem disorder

Regulation of CFTR through a SYK pathway

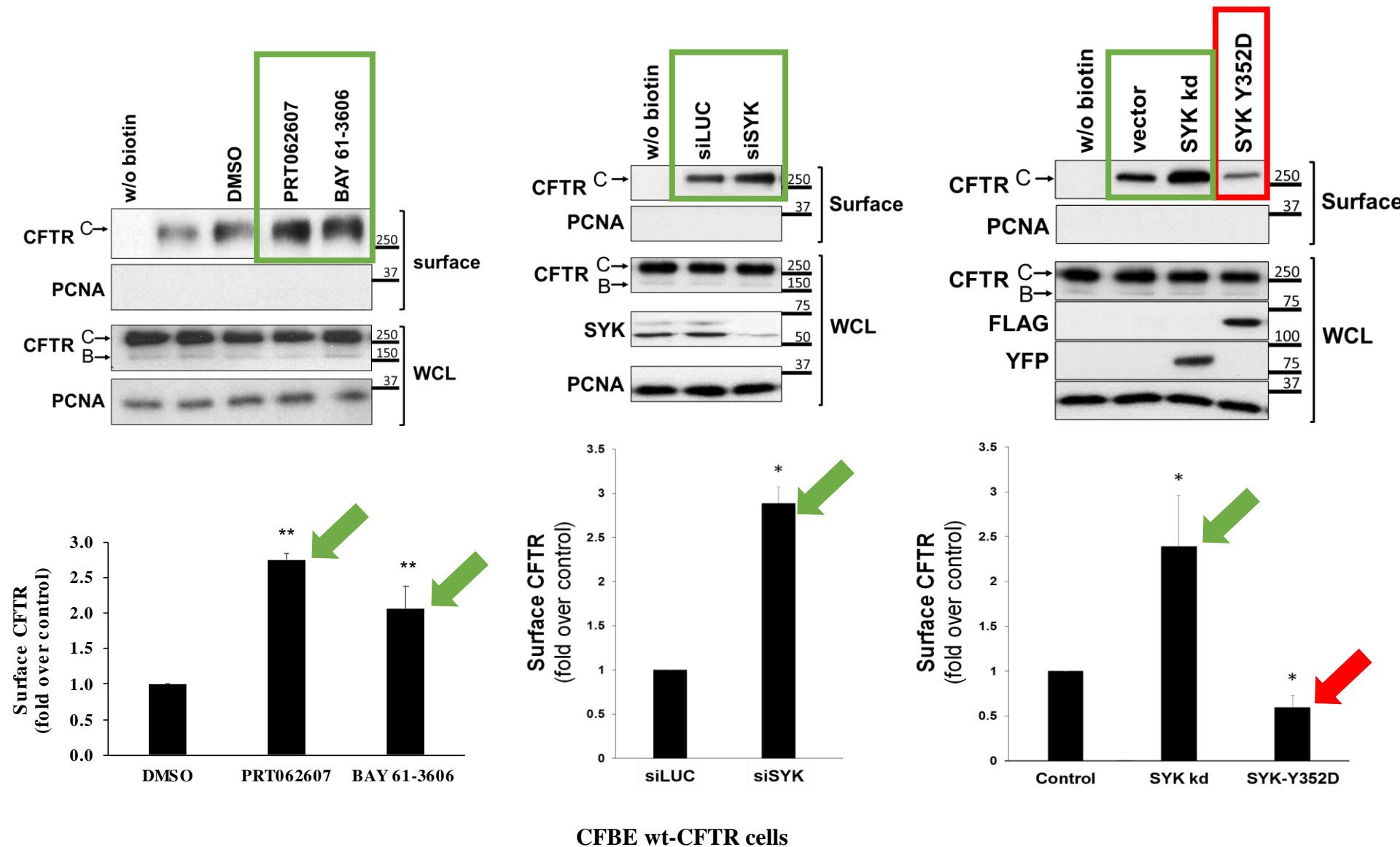


CFTR-transfected BHK-21 cells

Tyrosine kinase with pleiotropic effects in the human body

- Crucial role in adaptive immune receptor signaling
- Novel role in ion transport regulation

SYK activity modulates the cell surface expression of CFTR in human airway epithelial cells

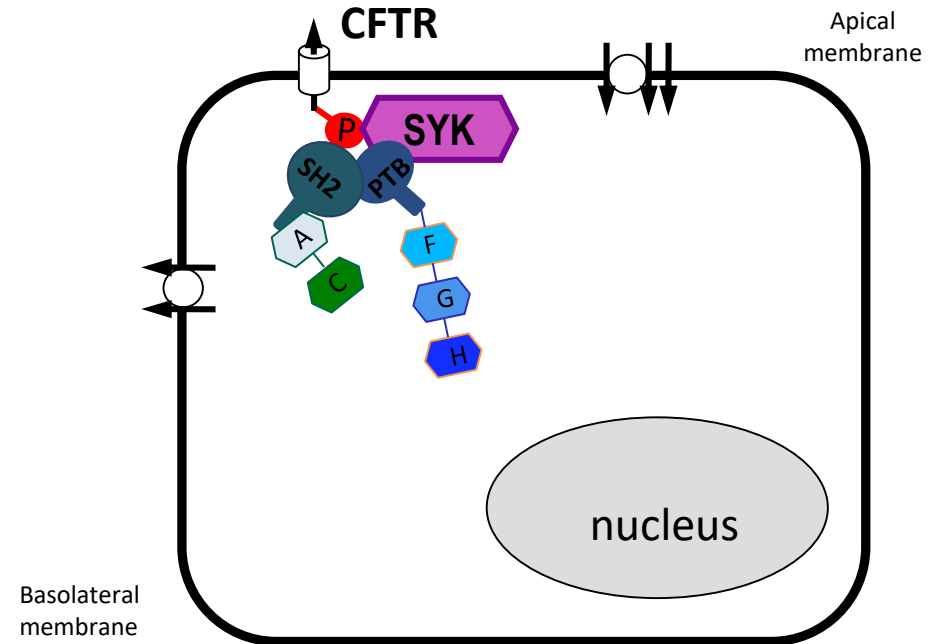


Identify phospho-tyrosine-binding proteins involved in the regulation of chloride transport protein and the underlying molecular mechanism

Tyrosine phosphorylation creates binding sites for proteins containing phospho-tyrosine binding domains (SH2 or PTB domains)



Isolate and identify adaptor proteins in the human proteome, which are able to recognize SYK-phosphorylated chloride transporting protein



Proteomic peptide-protein interaction screen

Schulze and Mann 2014
EGFR-Grb2 interaction

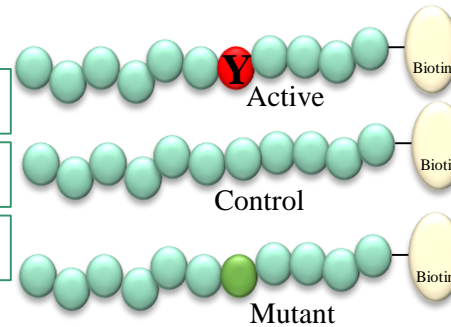
Biotinylated synthetic peptides

CFTR

IFGVS-**pY**-DEYRY

IFGVS-**Y**-DEYRY

IFGVS-**F**-DEYRY



SYK substrate motif
Y-D/E-D/E-X



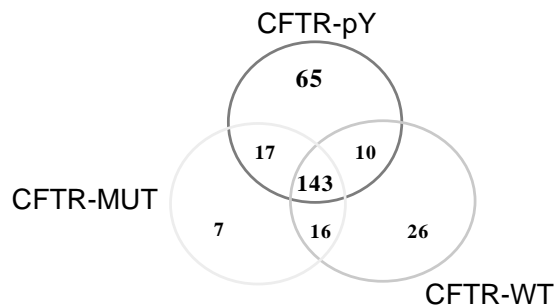
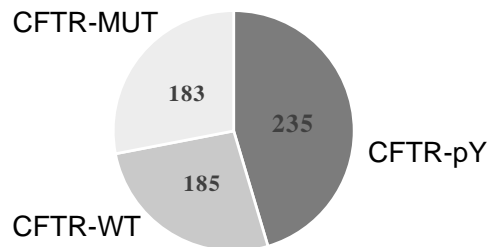
CFBE WT-CFTR cells

Mass
spectrometry

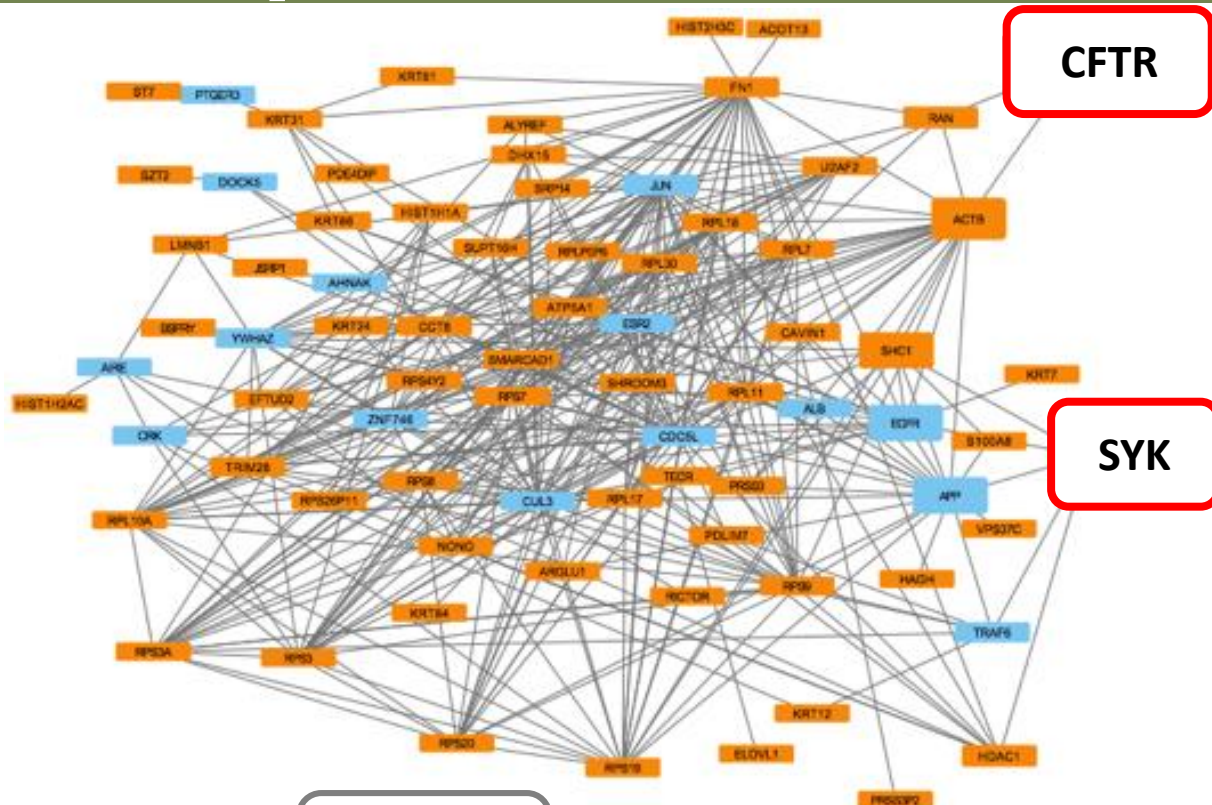


Bioinformatic analysis of MS data identifies a network of CFTR-associated proteins potentially involved in the regulation of chloride transport

603



61
CFTR-pY



Francisco
Pinto

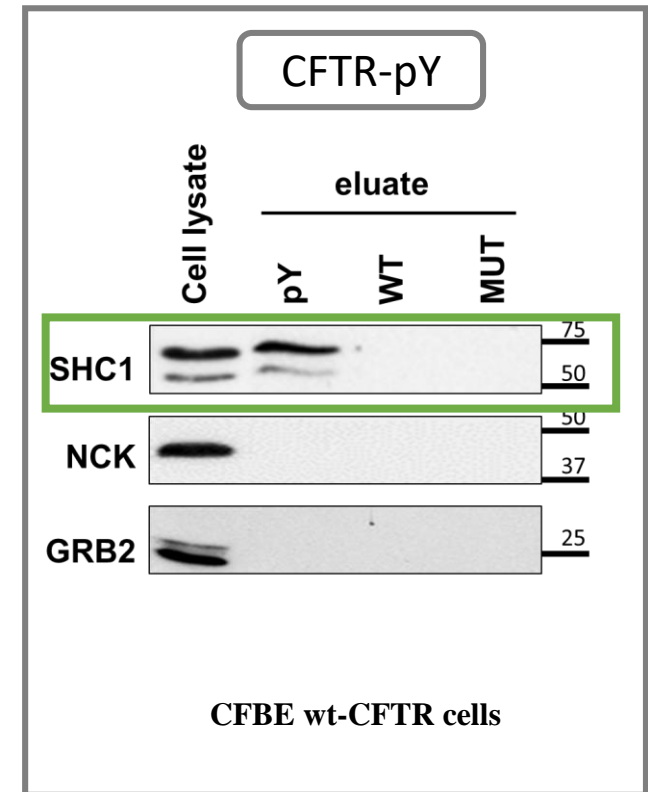


Paulo
Matos

SHC1 binds specifically to the CFTR phosphopeptide

12
CFTR-pY

Peptide	Accession number	Protein name
CFTR-pY	Q5W0U4	B box and SPRY domain-containing protein
	Q9NR12	PDZ and LIM domain protein 7
	Q9H4L7	SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A containing DEAD/H box 1
	Q96MG2	Junctional sarcoplasmic reticulum protein 1
	P29353	SHC-transforming protein 1
	Q9NWB6	Arginine and glutamate-rich protein 1
	P37108	Signal recognition particle 14 kDa protein
	Q9NRC1	Suppressor of tumorigenicity 7 protein
	Q9NPJ3	Acyl-coenzyme A thioesterase 13
	Q9BW60	Elongation of very long chain fatty acids protein 1
	Q8TF72	Protein Shroom3
	P98082	Disabled homolog 2
A5D8V6	Vacuolar protein sorting-associated protein 37C	

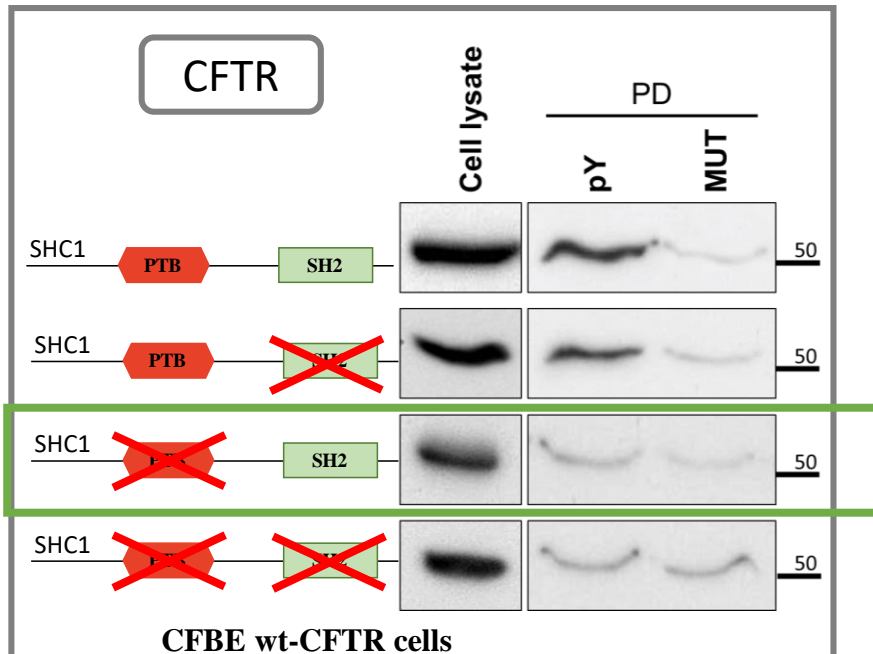
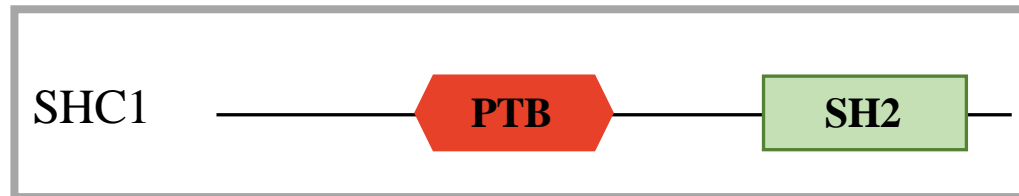


SHC1 binds specifically to the CFTR phosphopeptide

SHC1 binds to phospho-tyrosine in CFTR through its PTB domain

SHC1

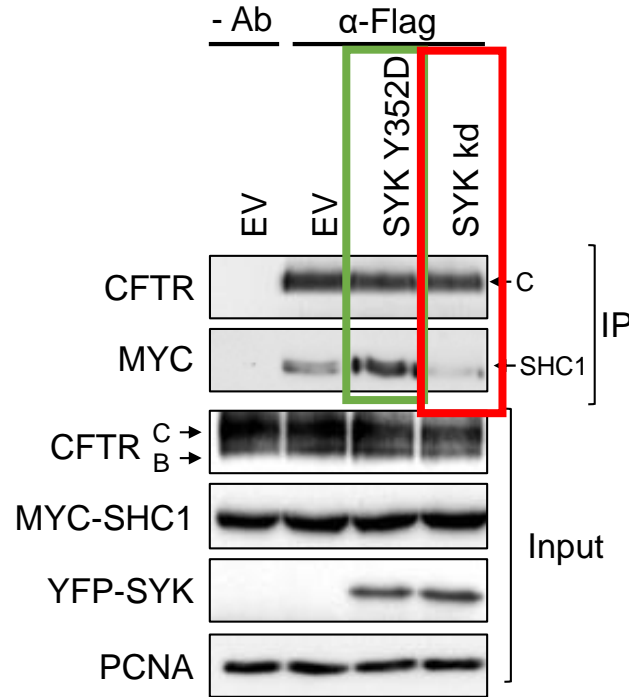
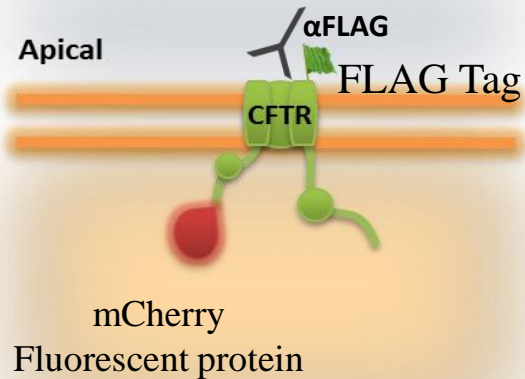
- Important role in activation of the RAS/MAPK pathway
- Unknown role in membrane protein traffic
- p46SHC1, p52SHC1 and p66SHC1



**Phosphorylated Tyr512
binds specifically to the SHC1
PTB domain**

SHC1 can associate with full-length CFTR

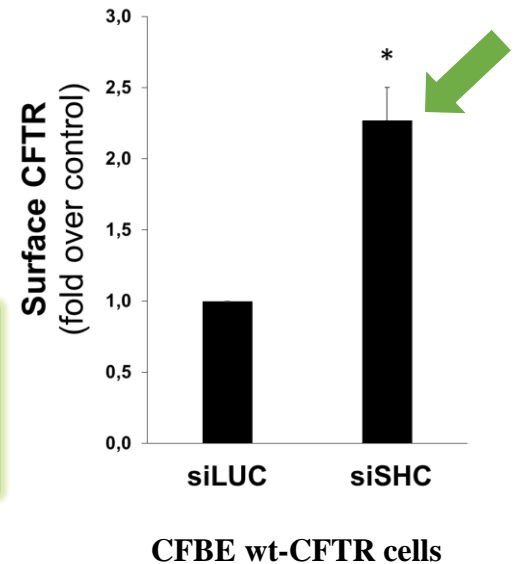
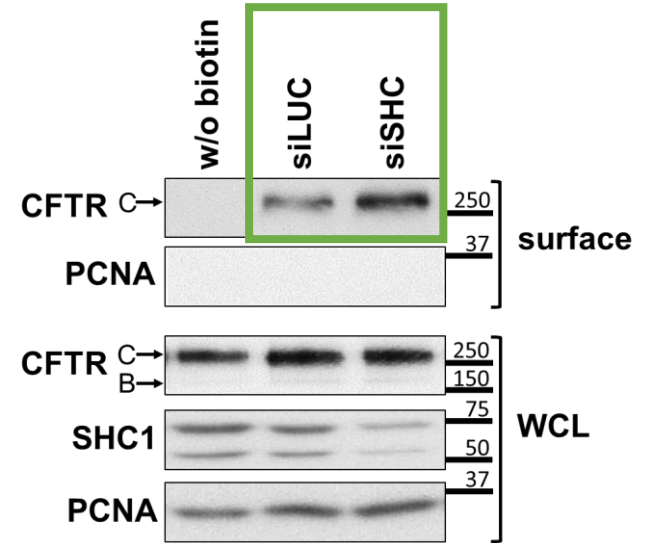
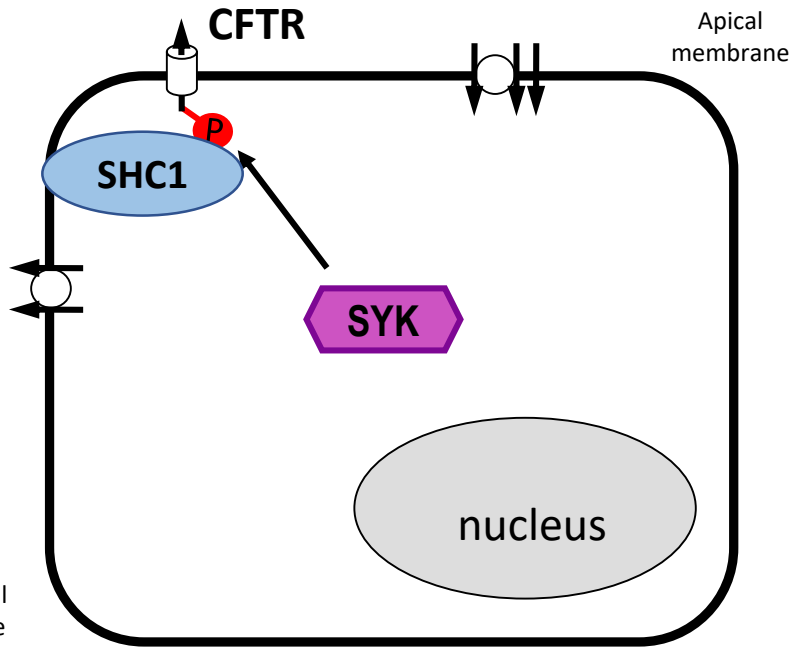
CFBE cell line stable transfected with
DOX inducible wt-CFTR



**SHC1 forms a complex with
CFTR that depends on the activity of
kinase SYK.**

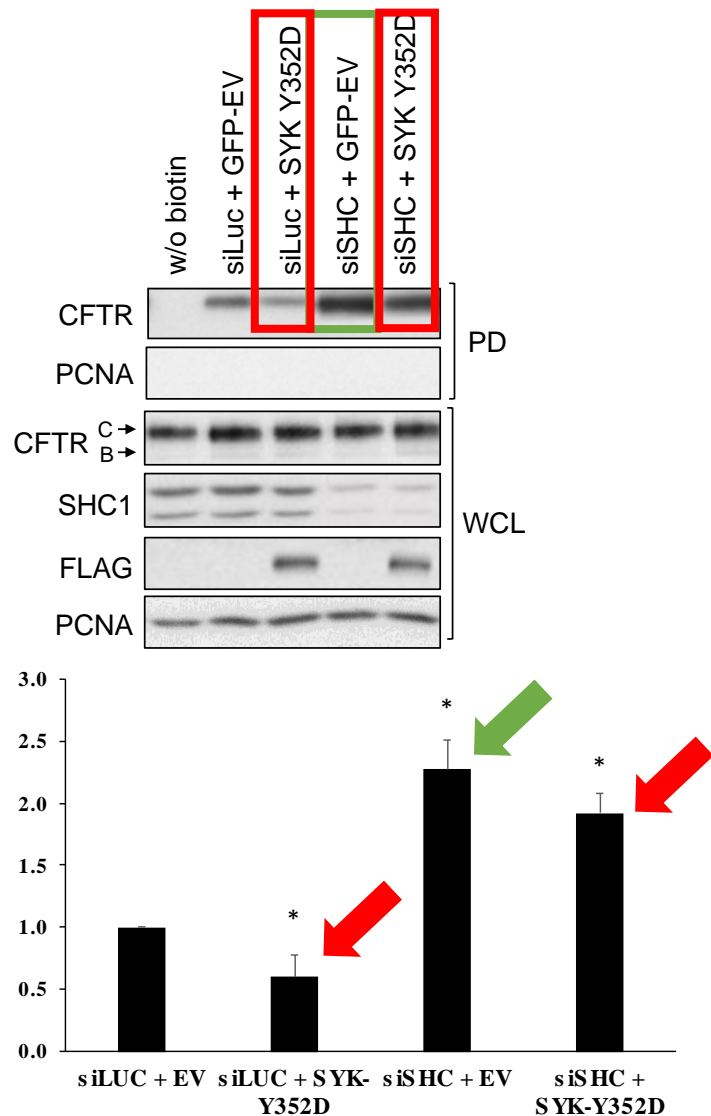
Kindly provided by Prof.
Margarida D. Amaral

Does SHC1 modulate CFTR cell surface expression ?

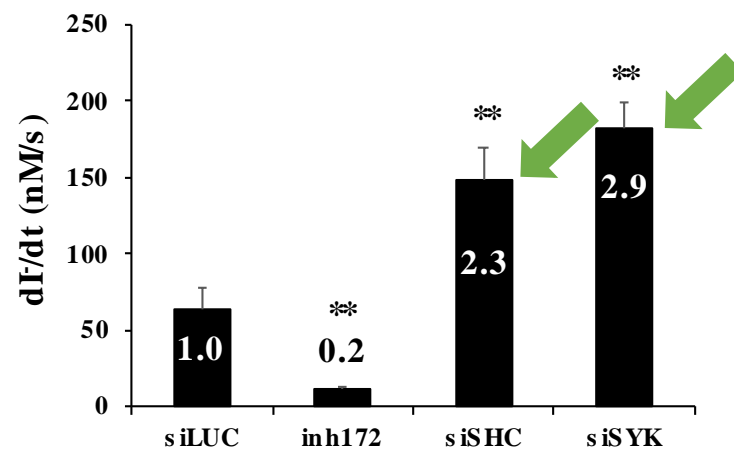
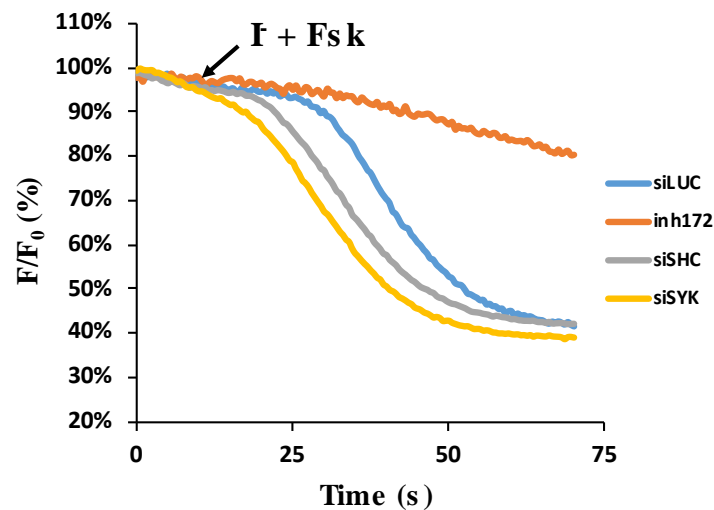


SHC1 modulates the amount of CFTR at the cell surface

SYK and SHC1 operate as a pathway and affect the cellular chloride transport activity



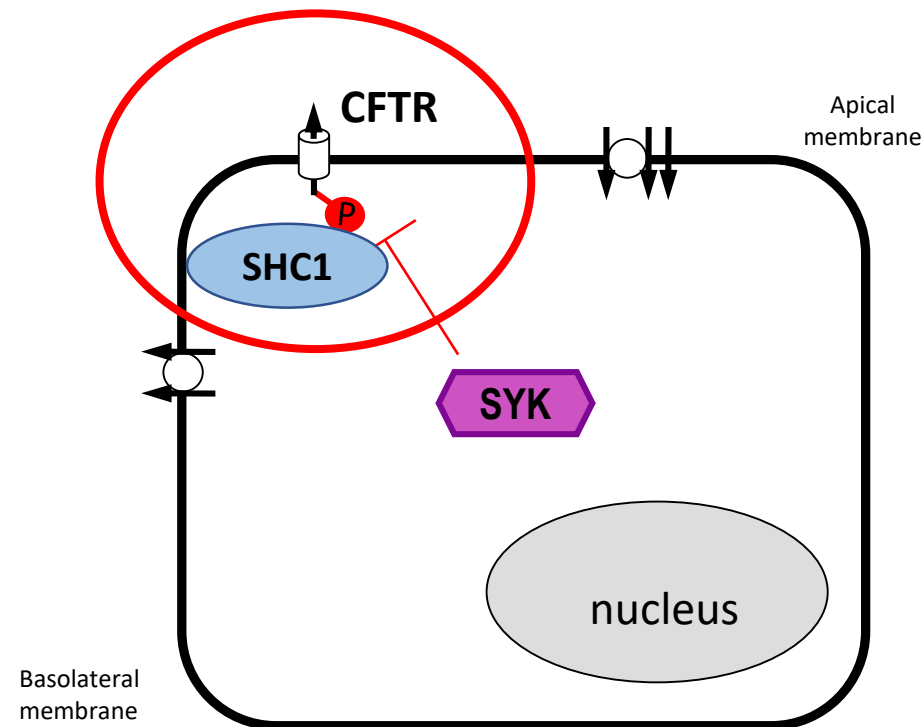
CFBE wt-CFTR cells



CFBE wt-CFTR cells stably expressing the HS-YFP sensor

Conclusions

- Tyrosine phosphorylation by SYK downregulates the cell surface abundance of the chloride channel protein CFTR;
- SHC1 was identified as a phosphotyrosine-binding regulator of CFTR;
- SHC1 forms a complex with CFTR at the plasma membrane upon activation of SYK and modulates the amount of cell surface CFTR



**Novel role for SHC1 in membrane traffic;
Potential biomedical implications for the identification of new
therapeutic targets in cystic fibrosis**

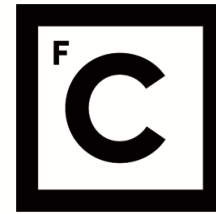
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