RARE DISEASE DAY
28 FEBRUARY

Università Politecnica delle Marche
Ancona
28 febbraio-1 marzo

Applicazioni “interactomiche” per la comprensione delle malattie rare: il caso della Fibrosi Cistica

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RARE DISEASES
(facts)

There are nearly 8000 rare diseases (some even without a name), it turns out that these "rare" diseases affect more than 300 million people worldwide (about 5-6% of the world's population). The economic cost of 379 rare diseases in the USA in 2019 was estimated to nearly 1 trillion USD .

94% of rare diseases lack an approved treatment

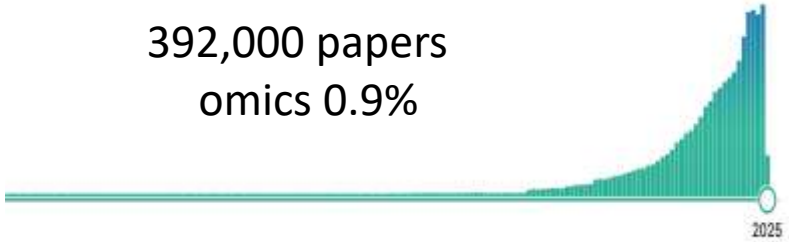
A large percentage of rare diseases are without diagnosis

Most of the known disease variants fall in coding regions of the genome, but much less is known about the role of noncoding region variants (90% of the eukaryotic genome) and structural variants in disease

RARE DISEASE DAY
28 FEBRUARY

RARE DISEASES (technologies)

392,000 papers
omics 0.9%



Transcriptomics, proteomics, phenomics, metabolomics, lipidomics and methylomics are already used, but epi-transcriptomics, metagenomics, fluxomics, glycomics and other omics are yet to be evaluated for the purposes of disease-gene prioritisation.

RARE DISEASES
(Challenges)

All the omics analyses rely on the systematic collection of well-organized large-scale datasets. Therefore, sharing patient-level data in public and controlled access repositories is highly relevant.

Despite these technological advancements, diagnosing rare diseases poses several challenges. Many clinicians struggle to interpret these novel findings, creating a demand for specialists who can translate results into clinical insights. Testing is often unavailable in low-resource medical settings, necessitating referrals to specialized centers. Omics, require sophisticated technologies, data repositories, bioinformatic pipelines, and age/tissue-matched controls, further complicating accessibility.

Cystic Fibrosis

Cystic fibrosis is the most frequent rare disease: around 4% of the population is a healthy carrier and around 200 new cases are recorded per year in Italy.

Cystic Fibrosis

- Autosomal recessive disease
- More than 2000 mutations in the gene coding for **CFTR**
- The deletion of Phenylalanine in position 508 (**ΔF**) present in 90% of patients worldwide

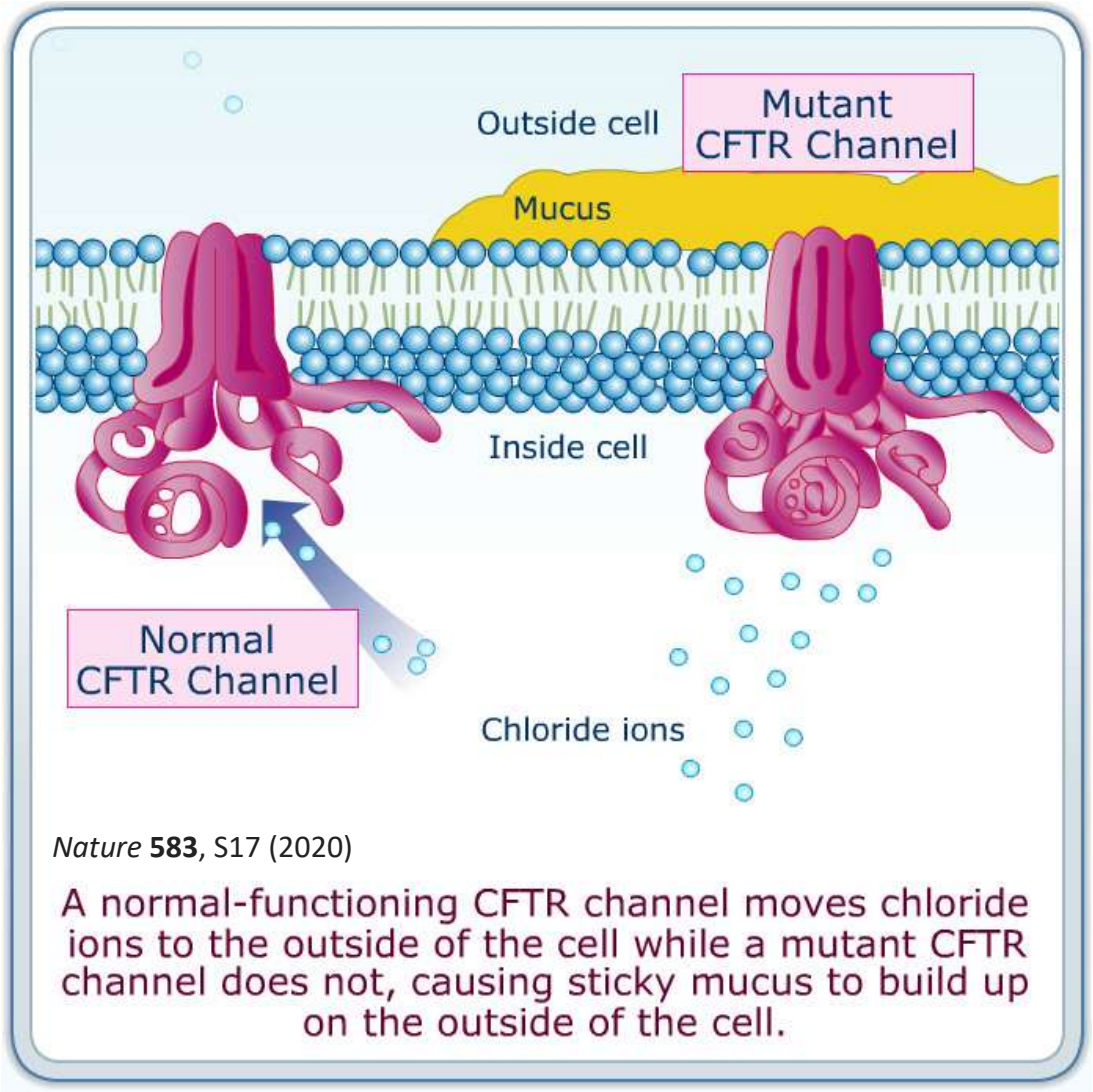
↓

bronchopulmonary obstruction and opportunistic infections (pseudomonas aeruginosa, mycobacterium)

Tissue transglutaminase activation modulates inflammation in cystic fibrosis .

Maiuri L et al. .**J Immunol.** 2008, 180(11):7697-705.

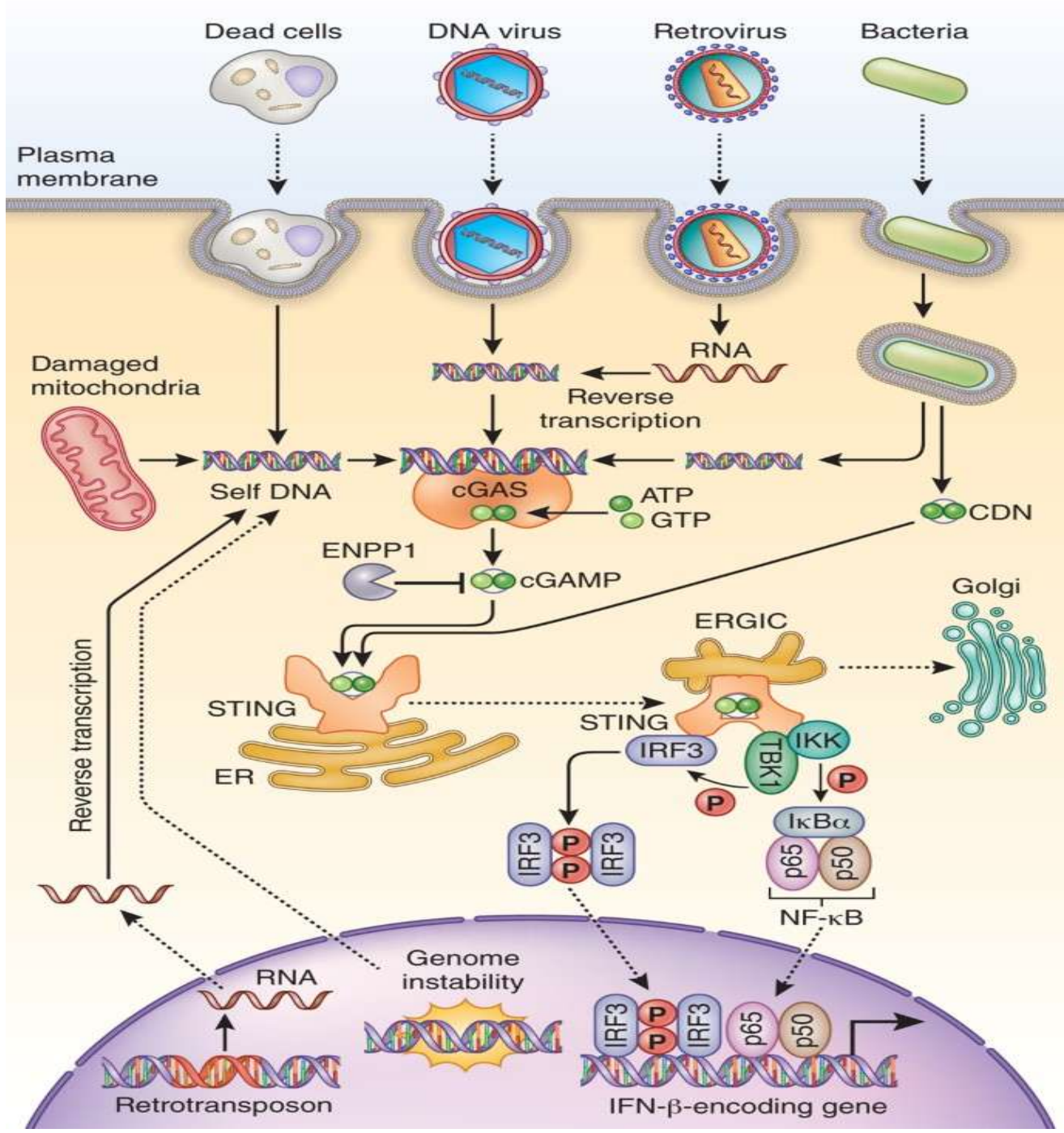
Transglutaminase 2 Regulates Innate Immunity by Modulating the STING/TBK1/IRF3 Axis.
Occhigrossi L, Rossin F, D'Eletto M, Farrace MG, Ciccosanti F, Petrone L, Sacchi A, Nardacci R, Falasca L, Del Nonno F, Palucci I, Smirnov E, Barlev N, Agrati C, Goletti D, Delogu G, Fimia GM, **Piacentini M.**
J Immunol. 206(10):2420-2429. 2021



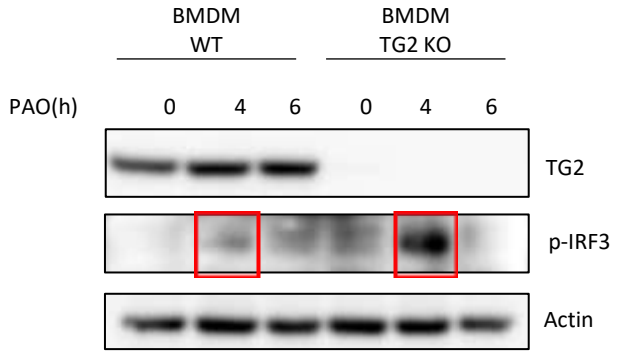
Nature **583**, S17 (2020)

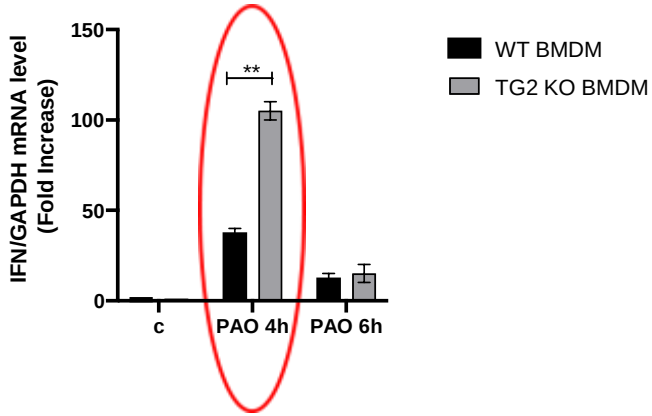
A normal-functioning CFTR channel moves chloride ions to the outside of the cell while a mutant CFTR channel does not, causing sticky mucus to build up on the outside of the cell.

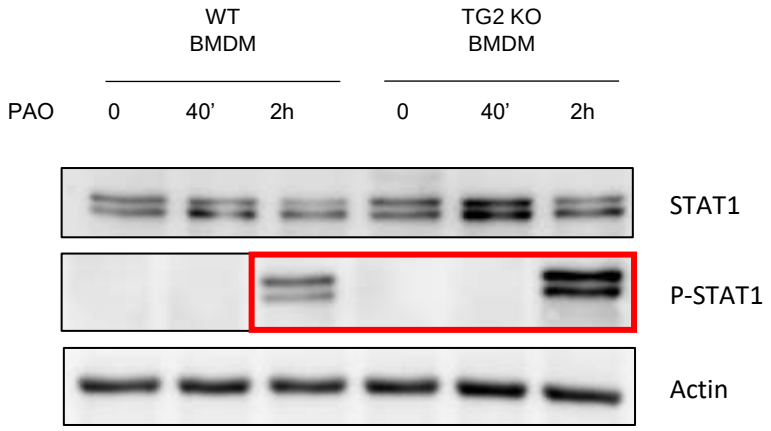
The cGAS/STING/TBK1/IRF3/IFN-β innate immunity pathway



The absence of Transglutaminase type 2 (TG2) in BMDMs infected with Pseudomonas aeruginosa (PAO) leads to increased levels of p-IRF3 and IFNβ

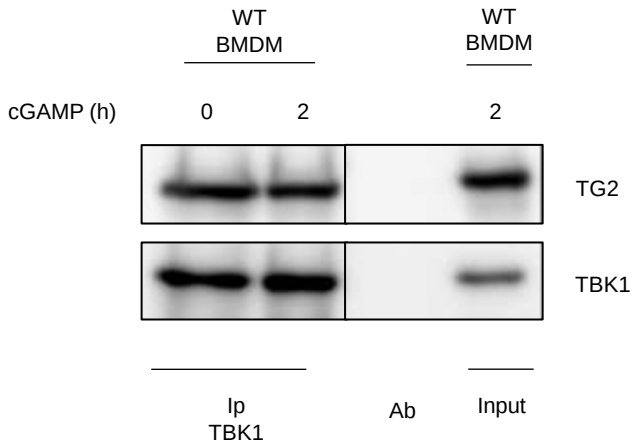


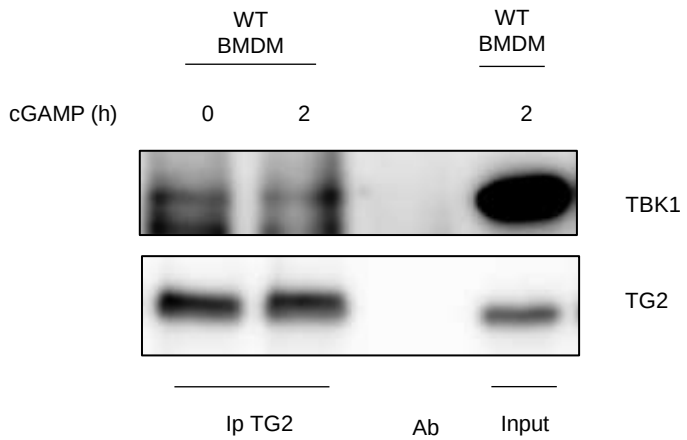


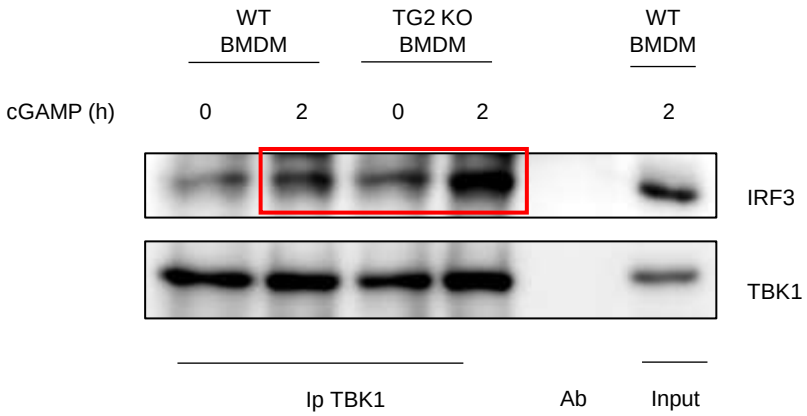


the cGAS/STING pathway is upregulated in TG2 KO BMDMs

TG2 interacts with TBK1 and this interaction limits the interaction between IRF3 and TBK1 in BMDMs



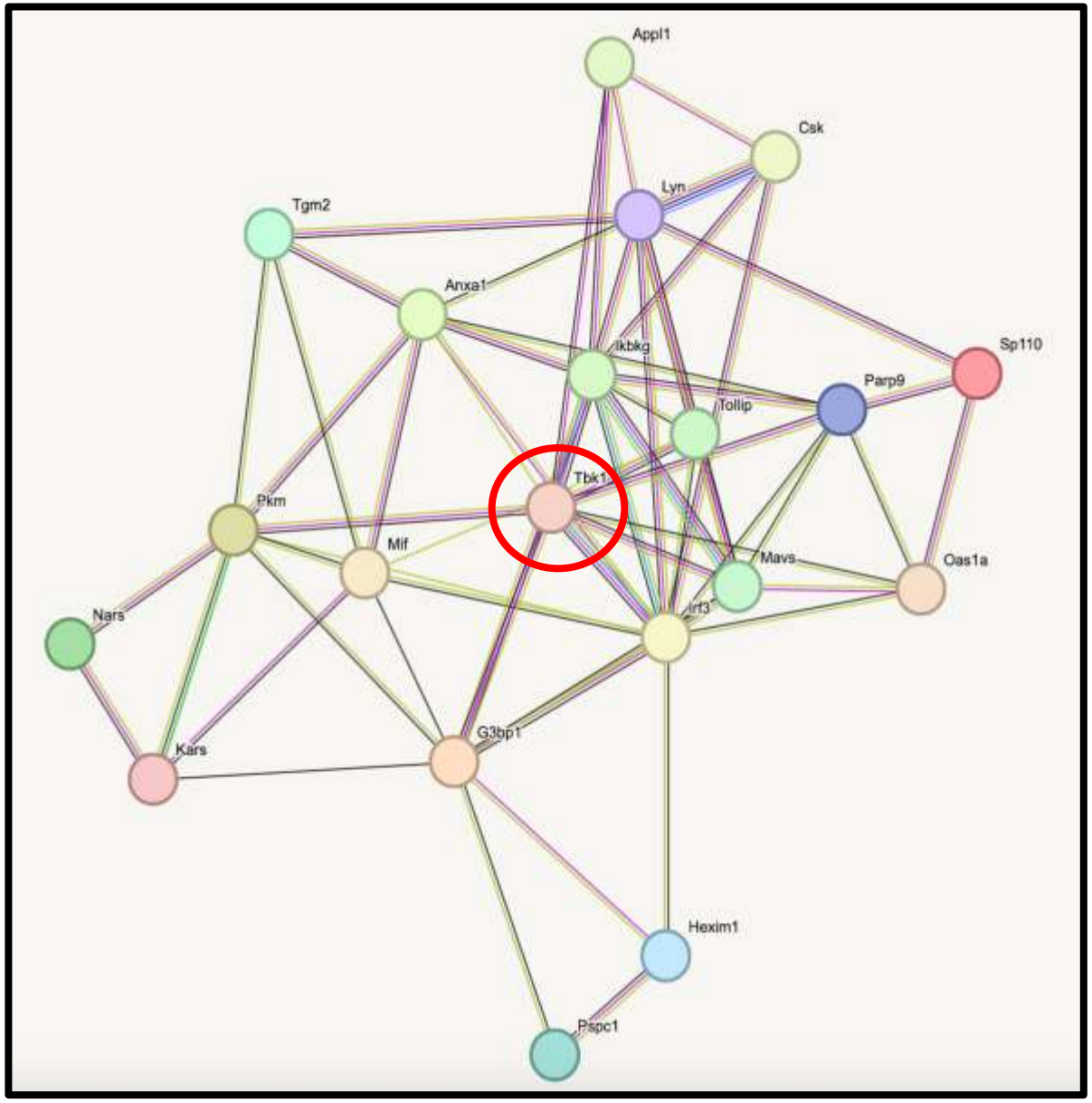




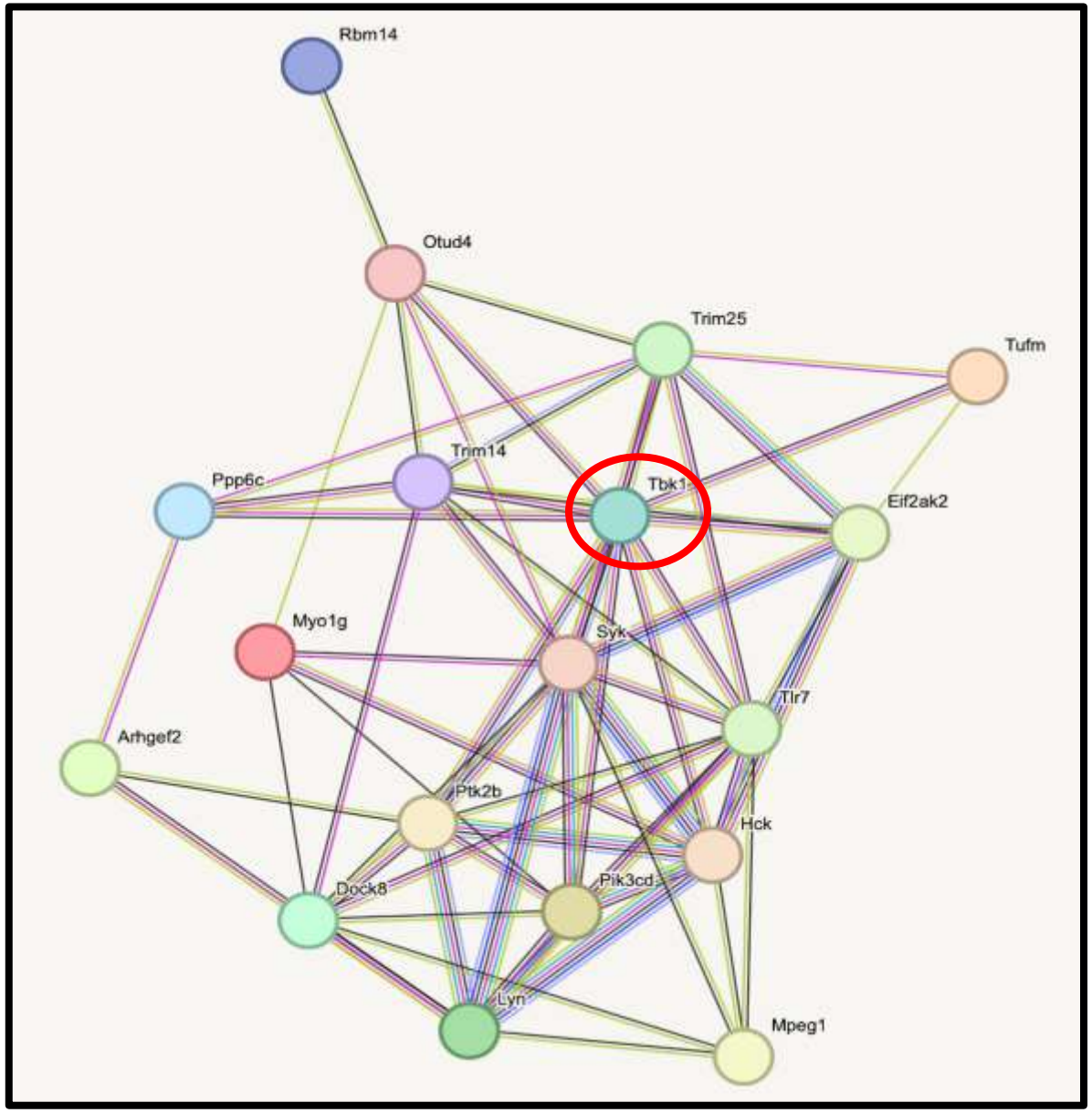
TG2 reduces the interaction of TBK1 with IRF3

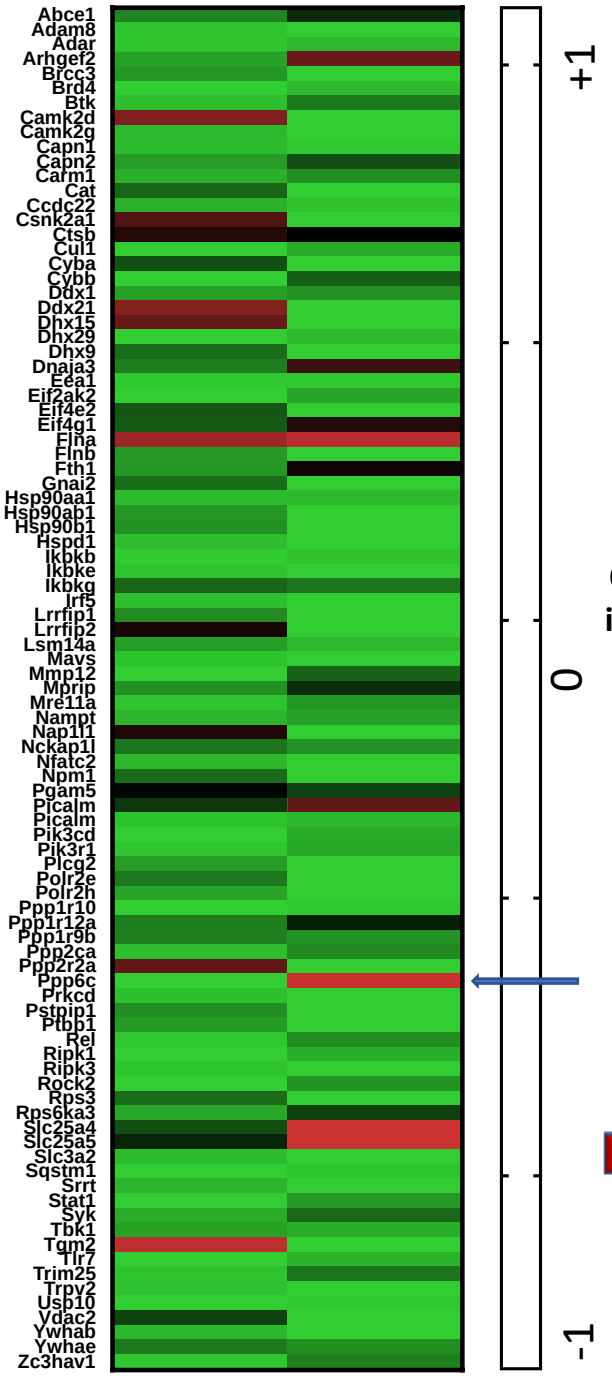
TBK1 interactome

plus TG2



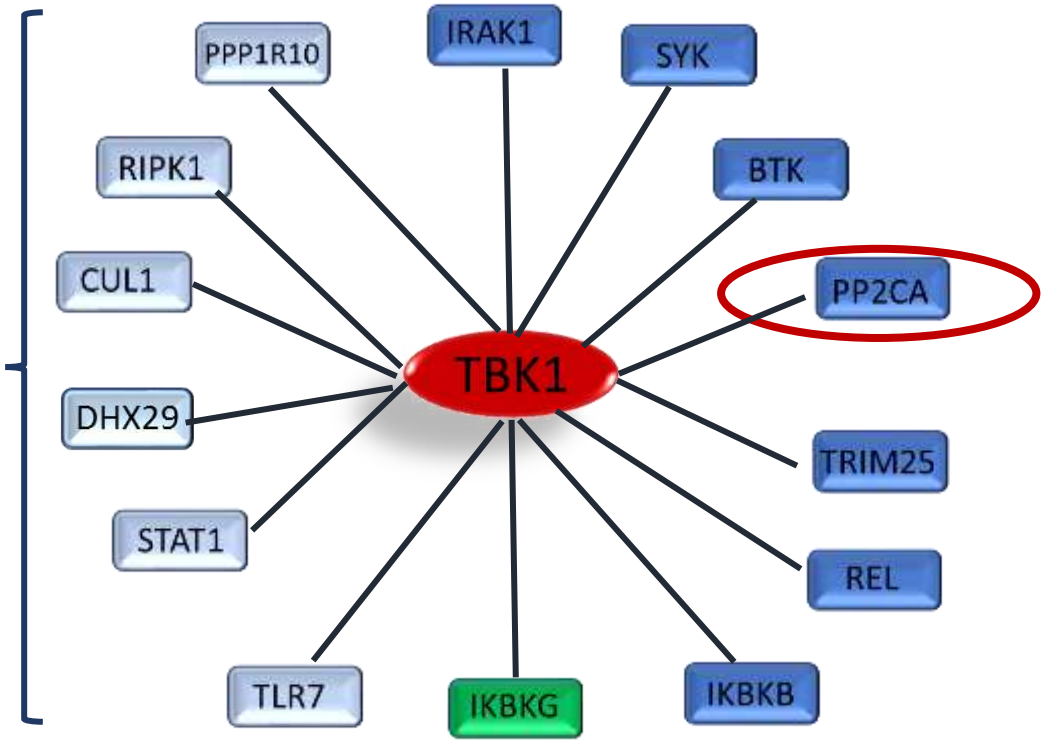
minus TG2





Only in KO

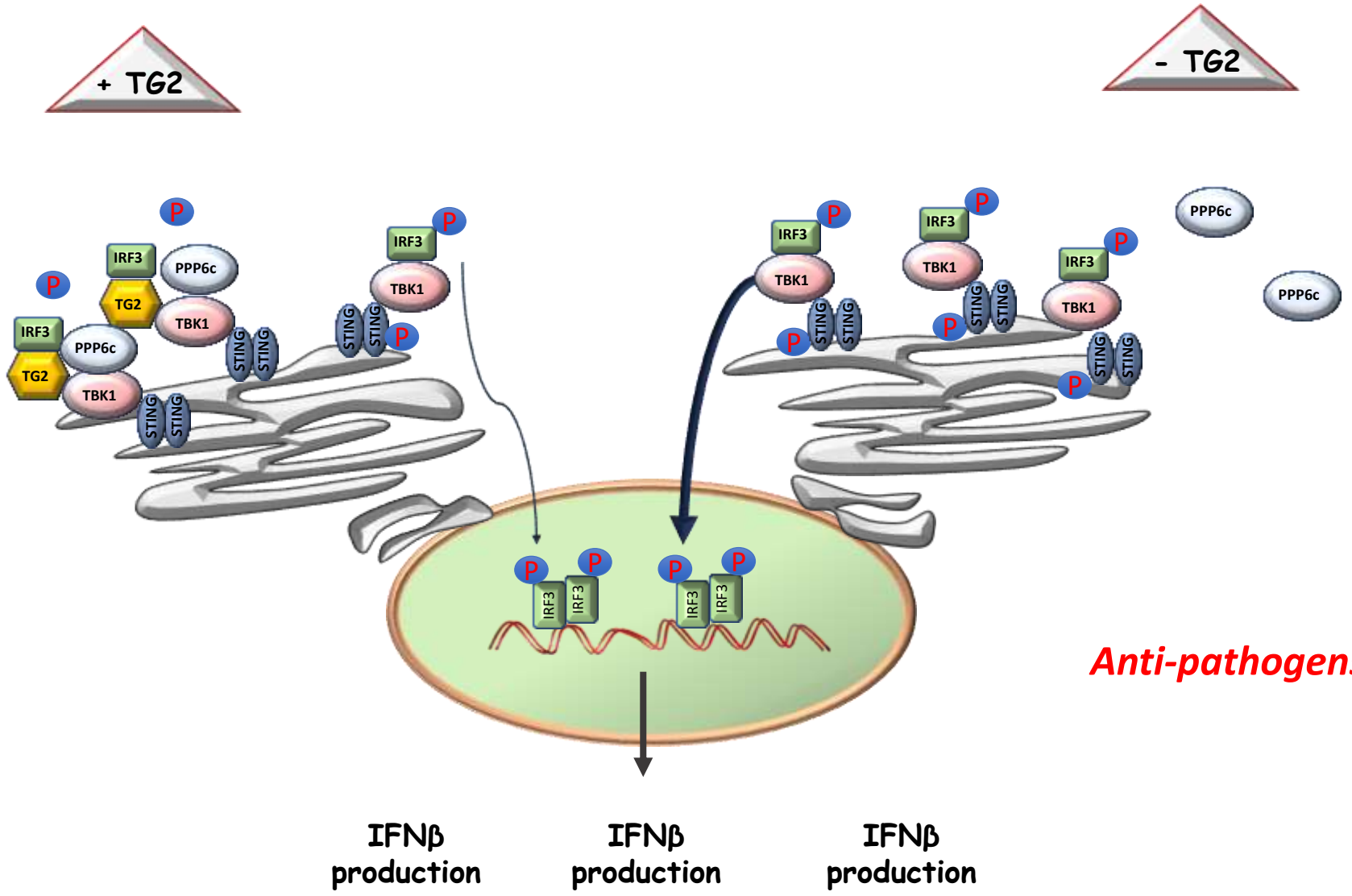
The absence of TG2 influences the TBK1 interactome



Particularly interesting is the drastic decrease of the phosphatase PPP6C that is the regulatory subunit of the PP2CA which is well known to dephosphorylate IRF3

PPP6C Negatively Regulates STING-Dependent Innate Immune Responses.
Ni G, Ma Z, Wong JP, Zhang Z, Cousins E, Major MB, Damania B.mBio. 2020 Aug 4;11(4):e01728-20.

Working model



Anti-pathogens response

Work Hypothesis

TG2 negatively regulates the STING pathway

TG2 is upregulated in Cystic Fibrosis

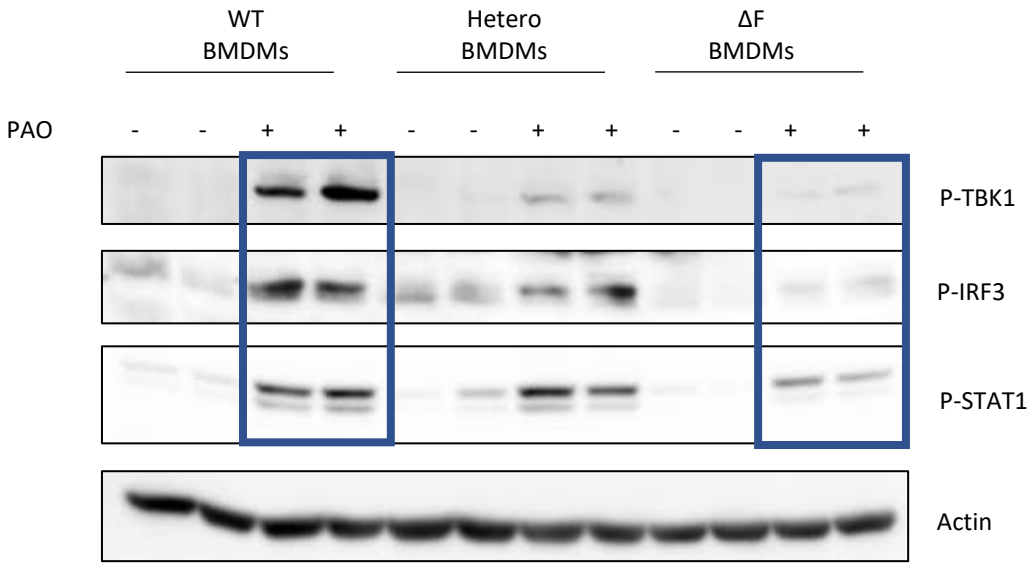
Is TG2 involved in CF by interfering with the STING/TBK1/IRF3 pathway ?

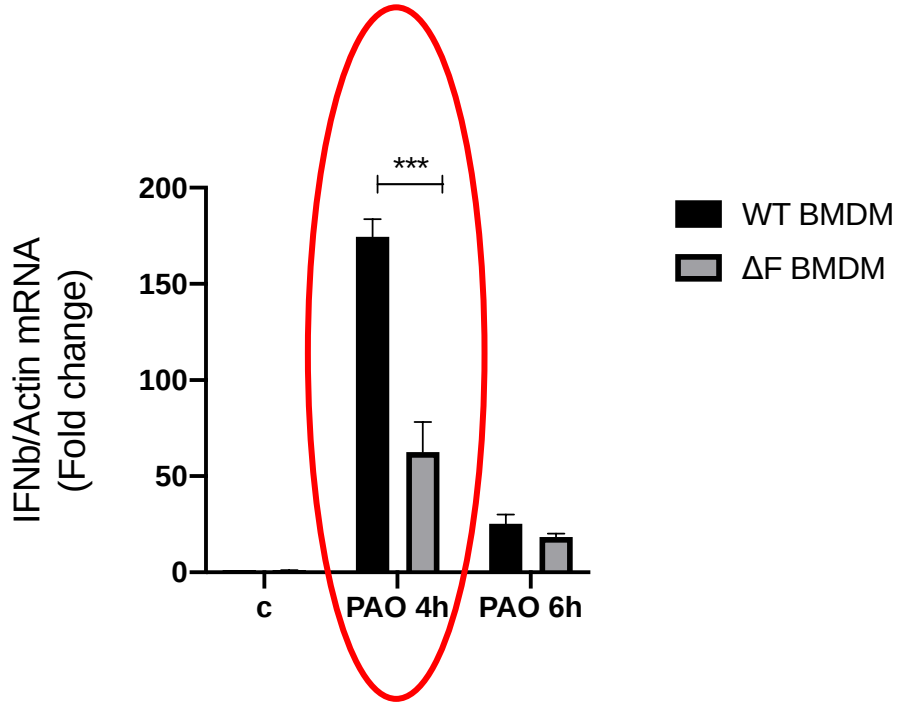
STING AGONISTS

is the STING axis impaired ?

Infection

Primary BMDMs from ΔF508del-CFTR mice infected with Pseudomonas aeruginosa do not activate the STING/TBK1/IRF3 pathway

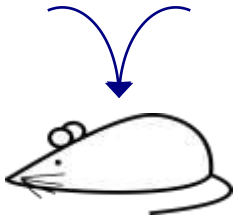




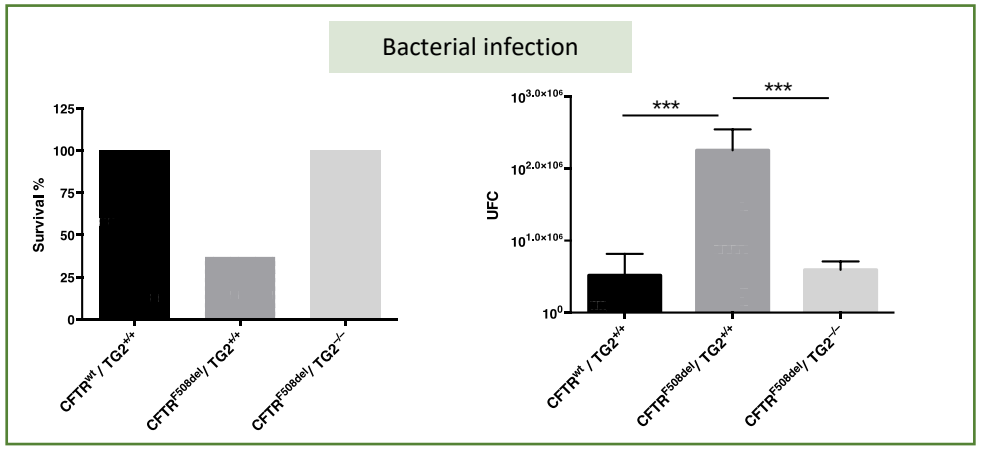
CF mouse model knock out for TG2

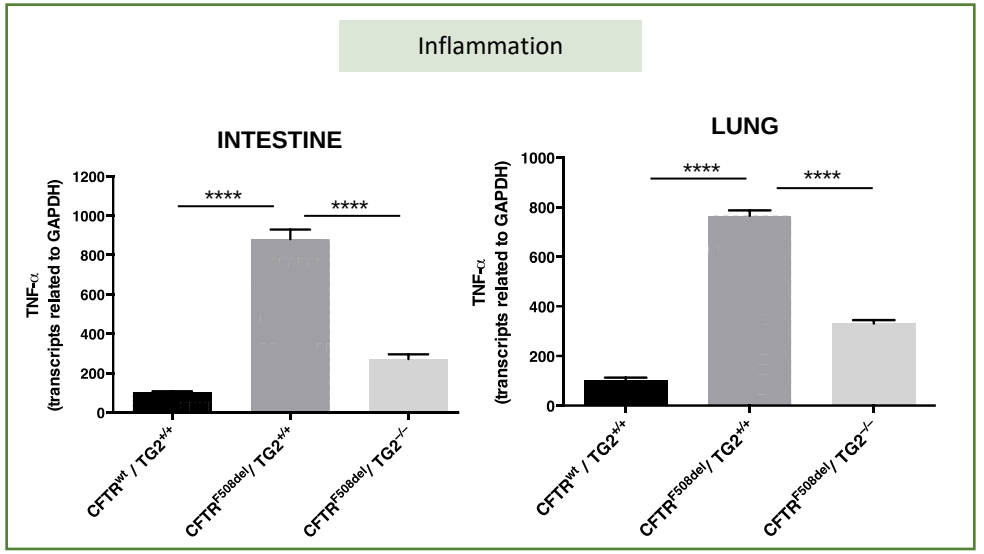
$CFTR^{wt/F508del}/TG2^{+/+}$

$CFTR^{wt/wt}/TG2^{-/-}$



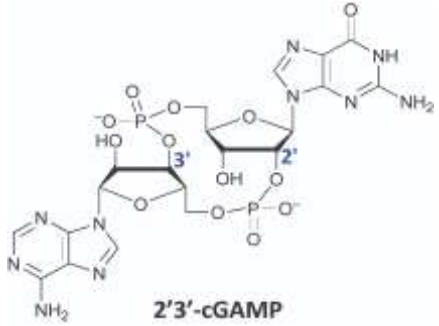
$CFTR^{F508del}/TG2^{-/-}$

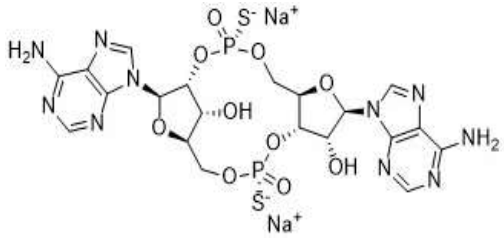




TG2 ablation in $CFTR^{F508del}$ mice re-establishes the immune response to the infection with *Pseudomonas Aeruginosa*.

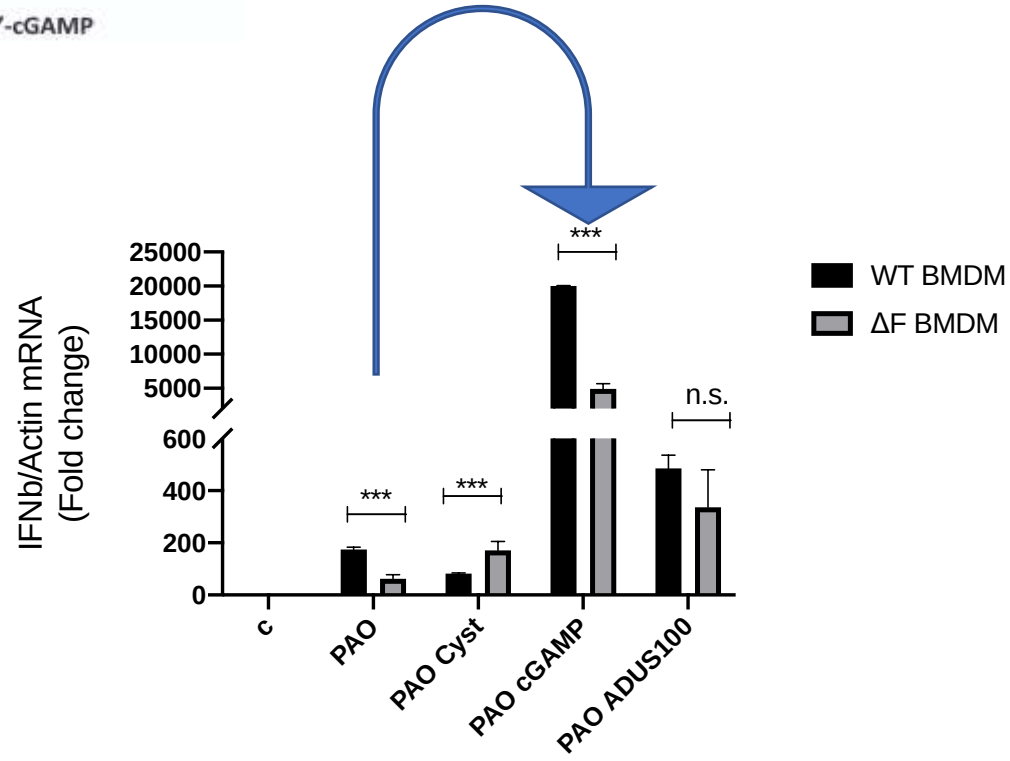
The STING agonists cGAMP and ADUS 100 rescue the INF-beta production and limit the infection in F508del-CFTR BMDMs infected with Pseudomonas aeruginosa

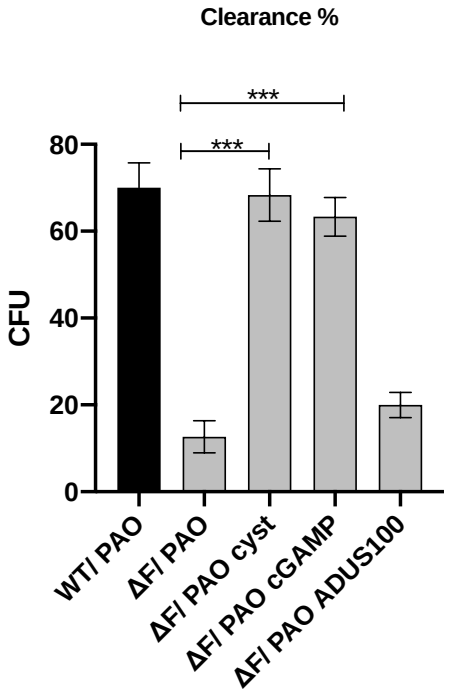




ADU-S100

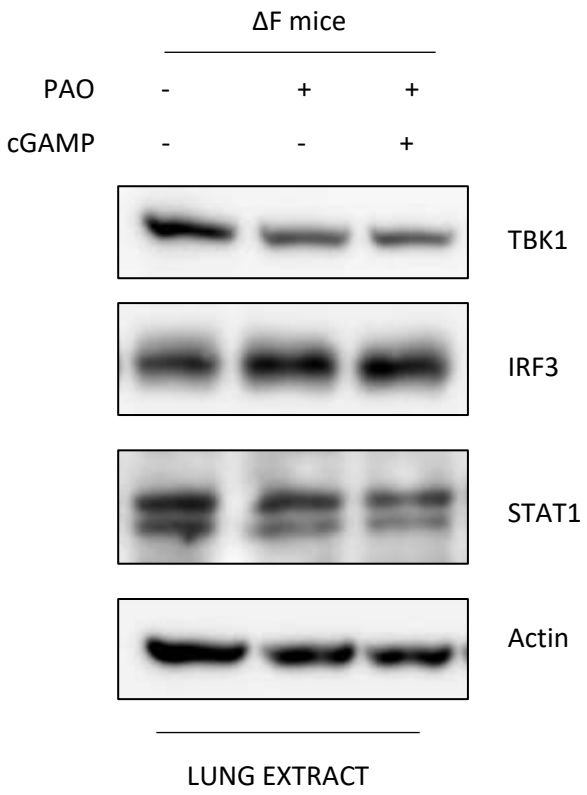
cGAMP favours PAO internalization and clearance by BMDMs from ΔF508 mice BMDMs

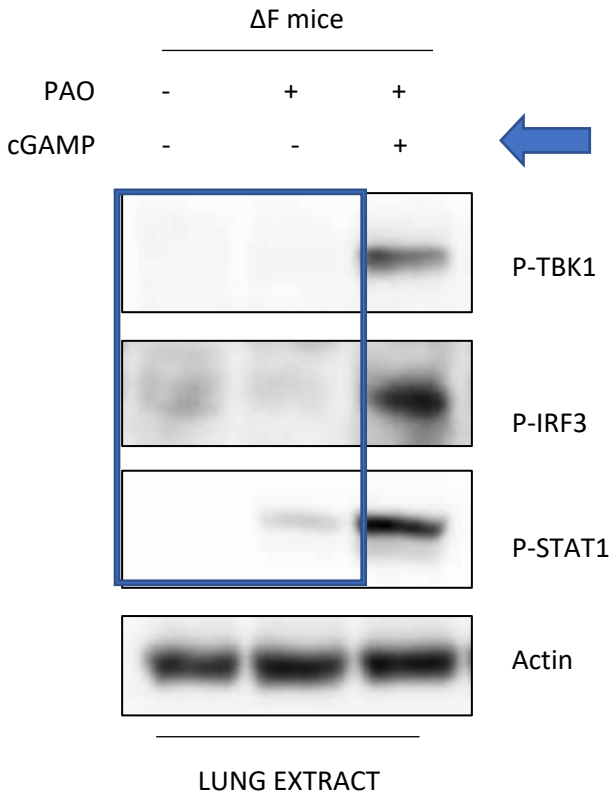


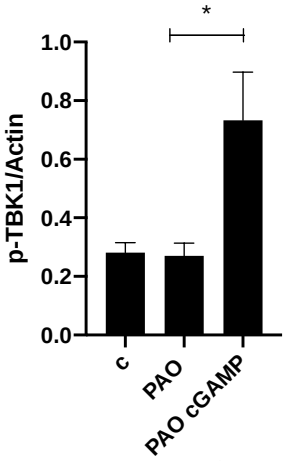


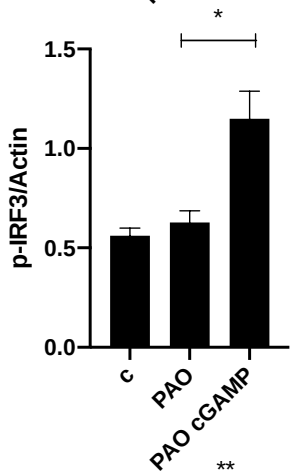
What happens in vivo in infected ΔF 508del-CFTR mice?

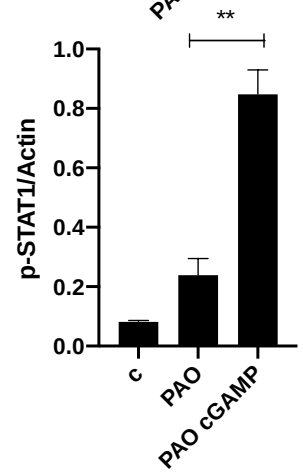
The STING agonist cGAMP rescues the INF-beta production in ΔF508del-CFTR mice infected with Pseudomonas aeruginosa via the STING/TBK1/IRF3-dependent pathway



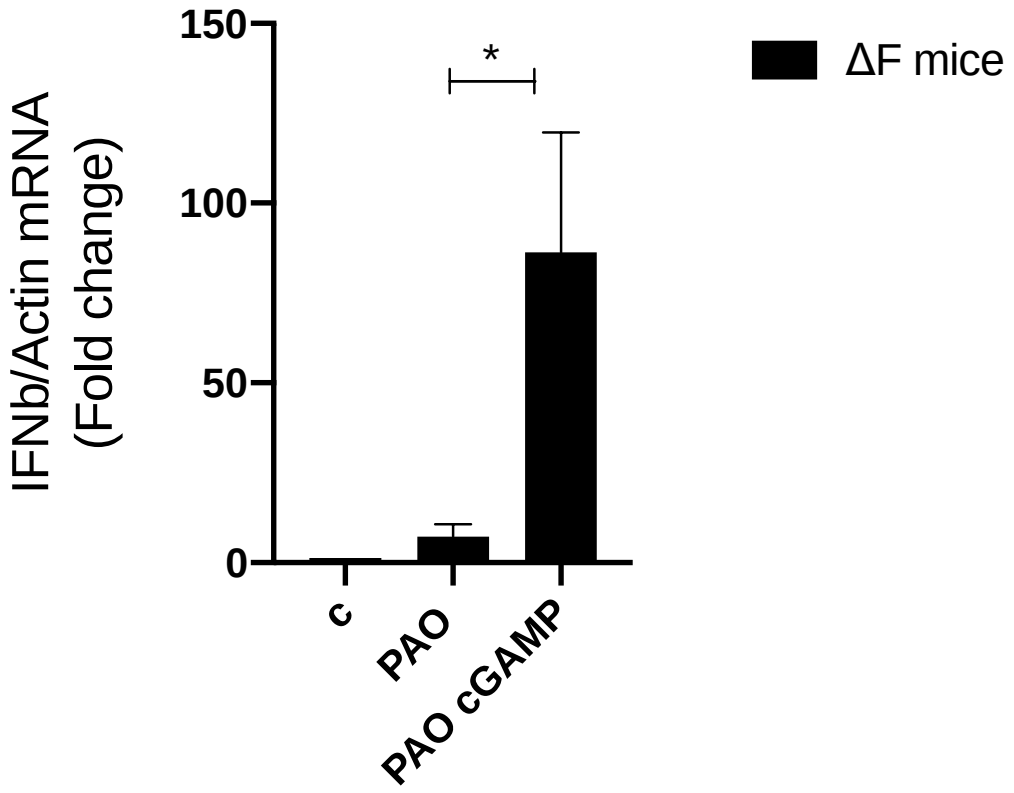


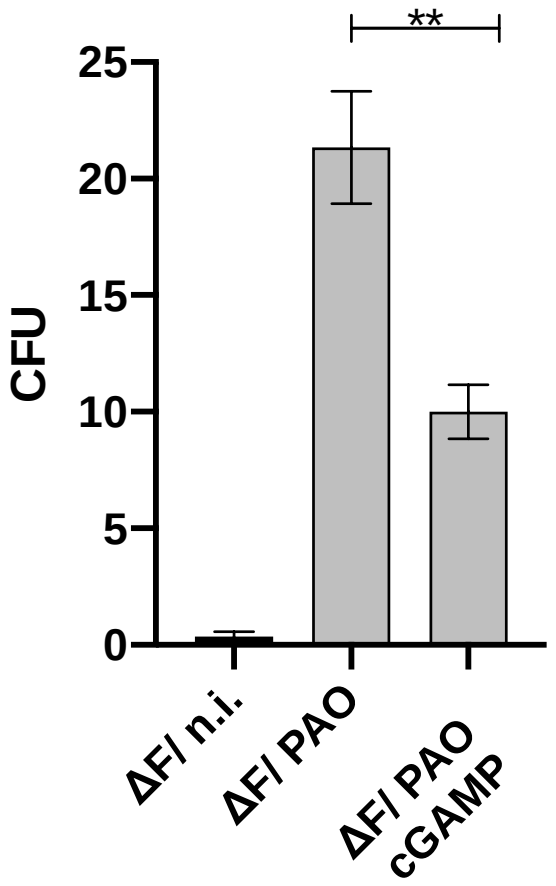




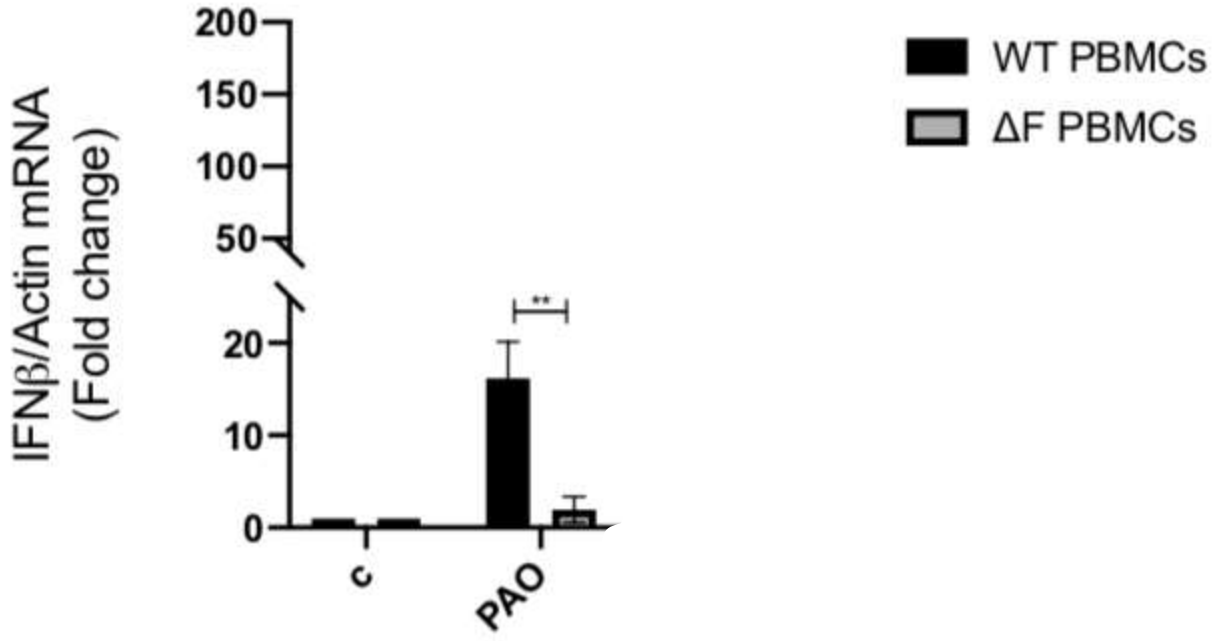


The STING agonist cGAMP rescues the INF-beta production and increases the clearance of Pseudomonas aeruginosa in the lung of F508del-CFTR mice





Is the STING agonist cGAMP able to rescue the INF-beta production in primary macrophages from Cystic Fibrosis F508del-patients?



CONCLUSIONS

- The innate immunity STING/TBK1/IRF3/IFNβ pathway is defective
  in Cystic Fibrosis.

- STING agonists and TG2 ablation can re-establish the production of IFNβ and the clearance of *Pseudomonas aeruginosa in vivo in ΔF508del mice.*

- The STING agonists can re-establish the production of IFNβ *ex vivo*
  in the PBMCs of Cystic Fibrosis ΔF508del patients.

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