CAT-5571 as a novel therapeutic that reduces infection and controls inflammation in cystic fibrosis

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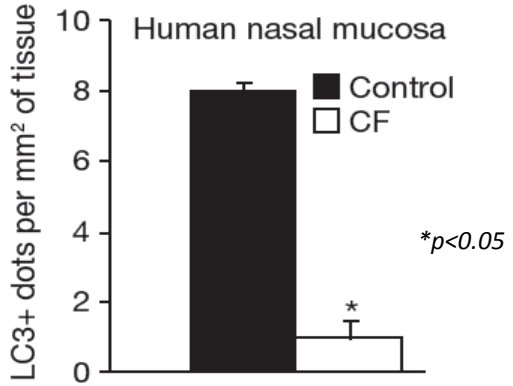
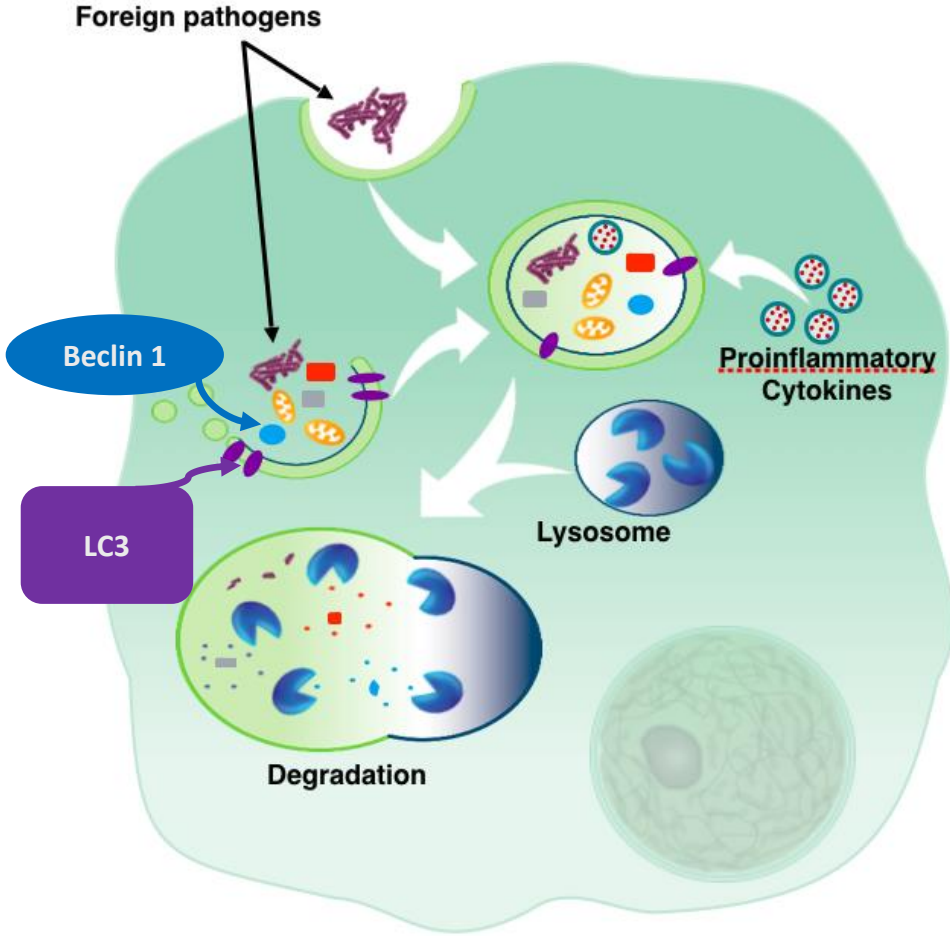
Autophagy

- **Depressed in cystic fibrosis**
- **Critical component of immune regulation and host defense**
- **Important for clearance of pathogens**

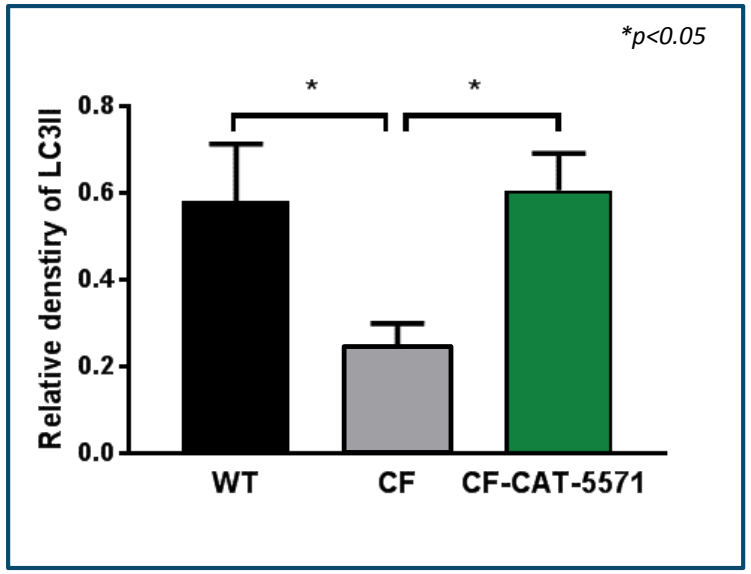


Human nasal mucosa from people with severe CF (n=10, homo or het ΔF508 CFTR)

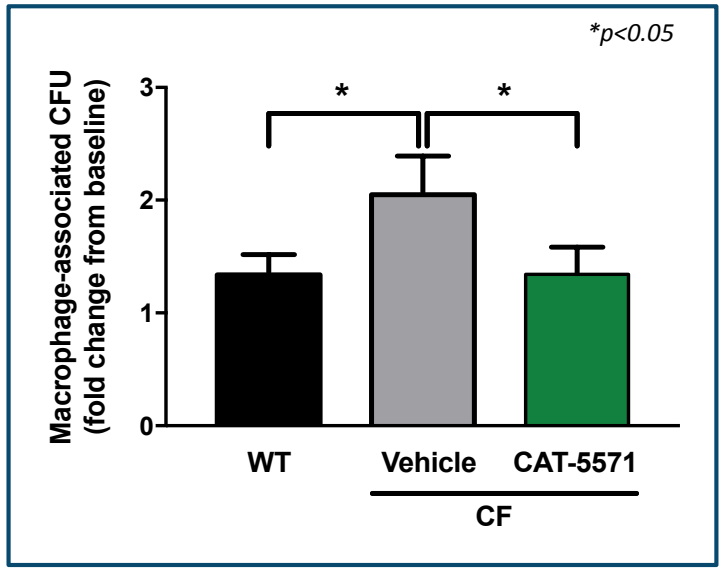
Luciani A, et al., Nat Cell Biol. (2010) 12: 863-75



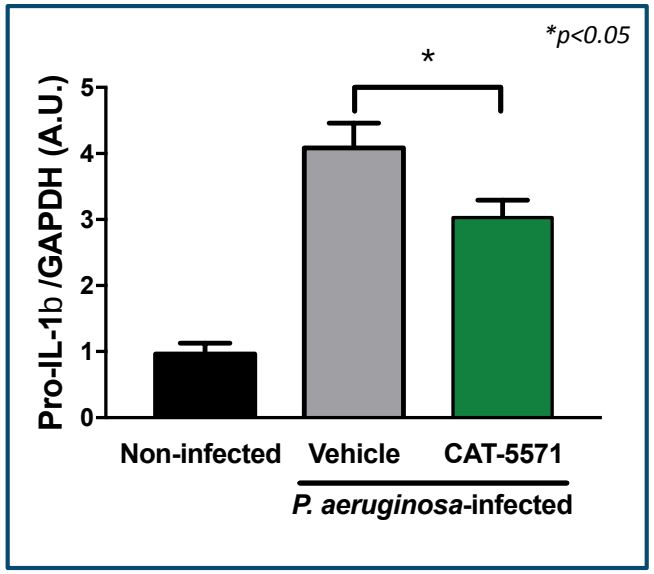
CAT-5571 restores LC3 in macrophages from ΔF508-CFTR mice to levels observed in wild type mice



CAT-5571 restores *P. aeruginosa* clearance in mouse ΔF508-CFTR macrophages



CAT-5571 reduces pro-IL-1β in *P. aeruginosa*-infected mouse ΔF508-CFTR macrophages

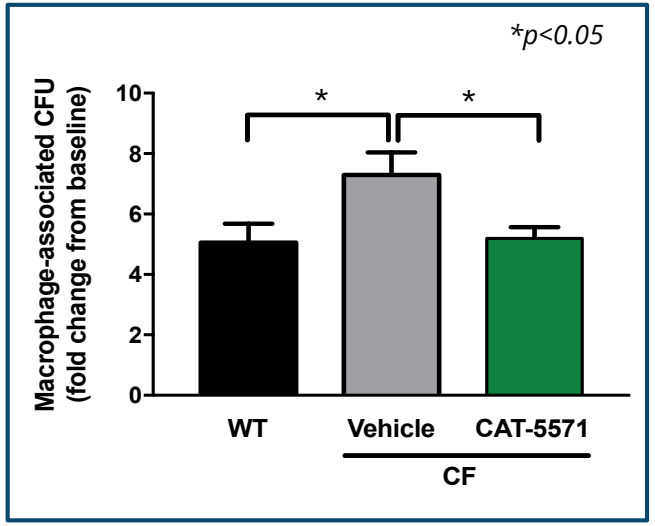


By restoring autophagy, CAT-5571 addresses a fundamental defect in CF that is present from birth

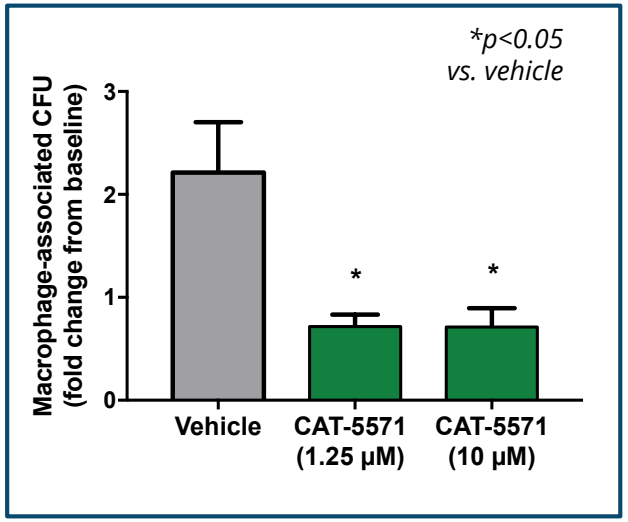
CAT-5571 enhances bacterial clearance and blunts the hyperinflammatory response in *P. aeruginosa*-infected macrophages

Bacterial clearance and IL-1β measurement in macrophages: WT and *cftr* $^{F508del/F508del}$ mouse macrophages were treated for 24 hours with vehicle or 10 µM CAT-5571, then infected with *P. aeruginosa* PA01 with an MOI of 10:1 for 2 hours . Elimination of extracellular bacteria was performed by replacement with media containing 200 g/mL gentamicin. The cells were then incubated at 37 °C in 5% CO_2 until lysis at 4 hours post infection. CFU were determined by serial dilution and plating onto nutrient agar. For pro-iL1β is 6 hours post infection. Cells lysates were analyzed by immunoblotting with anti-pro-IL1β antibody. Values are mean ± SEM of four independent experiments. Statistical analyses were performed using two-way ANOVA

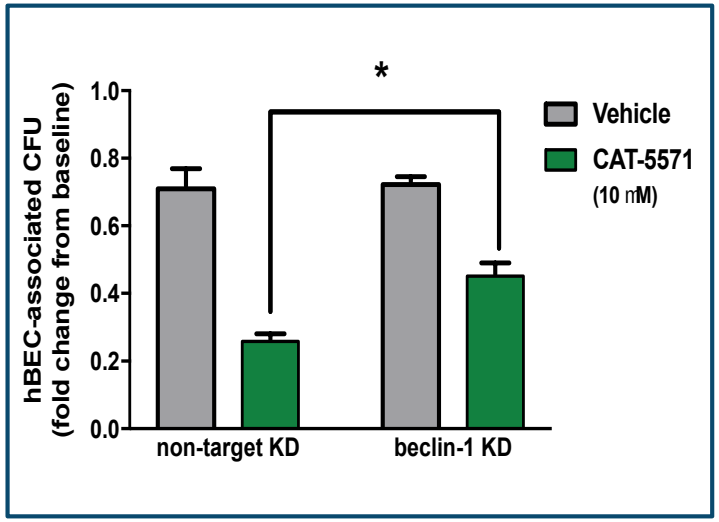
CAT-5571 enhances clearance of *B. cenocepacia* in mouse ΔF508-CFTR macrophages



CAT-5571 enhances clearance of *M. abscessus* in mouse ΔF508-CFTR macrophages



P. aeruginosa clearance by CAT-5571 was attenuated when autophagy was inhibited by beclin-1 knockdown in normal hBE cells



CAT-5571 enhances the clearance of multiple, difficult to treat pathogens affecting people with CF

CAT-5571's effect on pathogen clearance is mediated by beclin-1

Beclin-1 Knock down experiment: Normal human primary hBE cells were transfected either with beclin-1 siRNA or non-targeting siRNA for 20 hours. At 24 hours prior to infection, cells were pre-treated with CAT-5571 (10 µM) and the vehicle. Cells were infected with P. aeruginosa Xen05 72 hours post transfection at MOI of 1:50 in media containing CAT-5571 and vehicle control. Elimination of extracellular bacteria was performed at 2 hours post infection by replacement with media containing 200 g/mL gentamicin. The epithelial cells were then incubated at 37 °C in 5% CO_2 until lysis at 4 hours post infection. CFU were determined by serial dilution and plating onto nutrient agar. hBE-associated CFU relative to invasion (fold change). Values are mean ± SEM (n =3). Statistical analyses were performed using one-way ANOVA followed by multiple comparison test (* $p < 0.05$)

CAT-5571: Breaking the Downward Spiral of CF Progression

- **Novel Mechanism of Action**
  - Activates depressed autophagy, restoring host defense while preventing hyper-inflammation
  - Effective independent of CFTR mutation
- **Addresses Difficult to Treat Pathogens**
  - Pseudomonas
  - Burkholderia
  - Non-tuberculous mycobacteria
- **Host-Directed Therapy**
  - Potential to avoid typical bacterial resistance mechanisms
- **Acts in Concert with other CF Therapies**
  - Potential to augment efficacy of antibiotics
  - Potential to work on top of CFTR correctors and potentiators
- **Orally Administered**
  - Does not add to inhalational treatment burden