

“Microhemorrhage-associated tissue iron causes macrophage lysosomal leakage and loss, which increases the risk for invasive aspergillosis in a cystic fibrosis mouse model of airway transplantation”

Efthymia Iliana Matthaïou¹, Wayland Chiu¹, Carol Conrad², Joe Hsu¹

1. Department of Medicine: Division of Pulmonary, Allergy, and Critical Care Medicine, Stanford School of Medicine, 2. Department of Pediatrics: Division of Pulmonary Medicine, Stanford School of Medicine

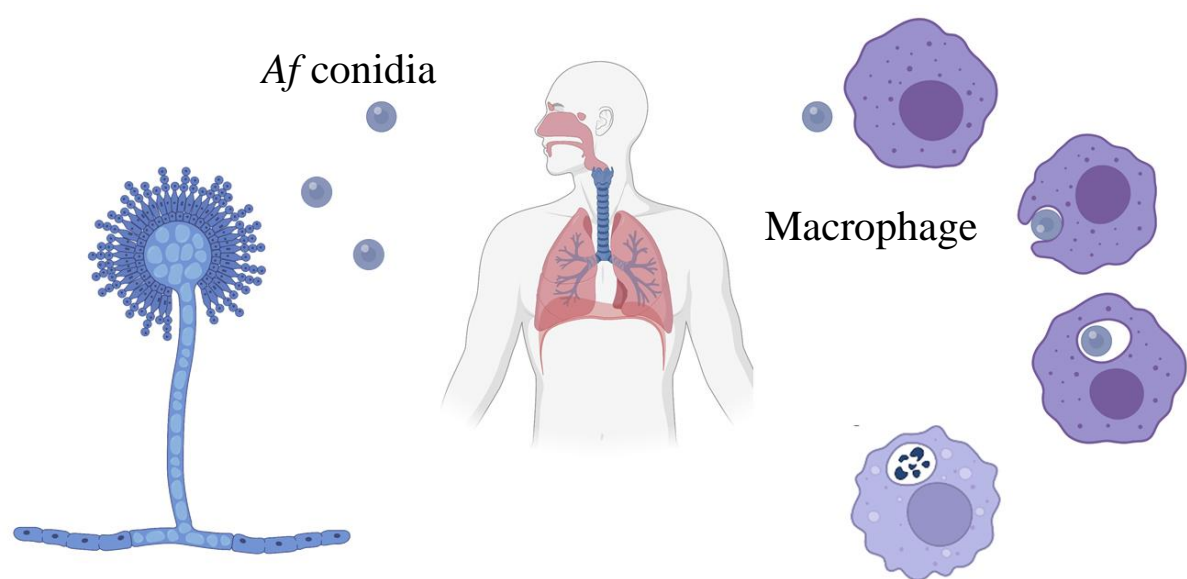


Introduction

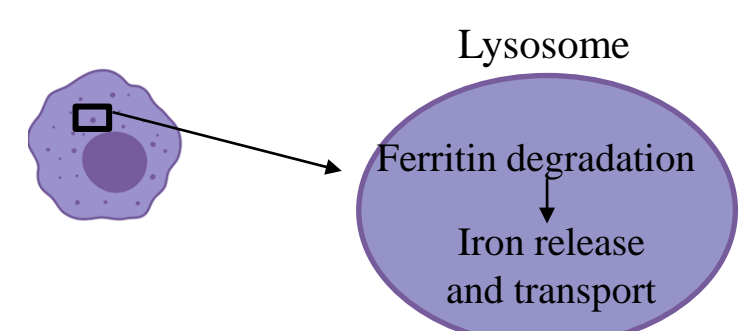
Aspergillus fumigatus (Af)-related pulmonary diseases are life-threatening, and occur in up to 30% of lung transplant recipients (LTRs). In cystic fibrosis (CF) LTRs the incidence of Af infections is higher than non-CF LTRs. Putative factors such as prior fungal colonization do not justify this increased risk. Defects in cystic fibrosis transmembrane conductance regulator (CFTR)-deficient macrophages (mφs) have been suggested to increase the risk of infections in CF patients. Using a murine orthotopic tracheal transplant (OTT) model, we have shown that iron-overload triggers Af invasion. We developed a murine CF OTT model (using a CFTR knockout (CFTR^{-/-}) recipient) and investigated how iron-overload affects CF mφ function. We hypothesize that iron-overload, in the CF transplant, impacts the ability of the CF mφ to control Af, promoting fungal invasion.

Background

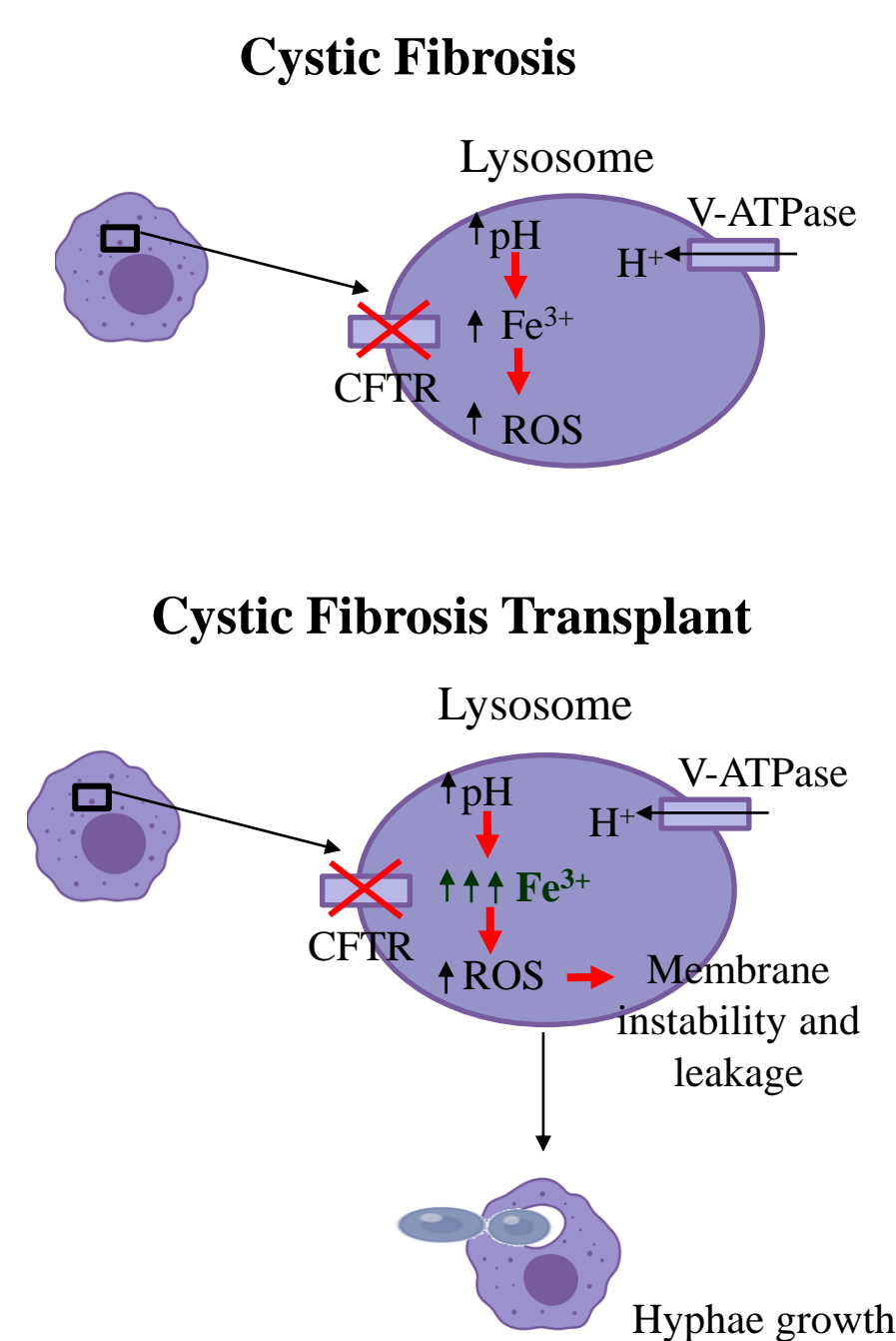
Macrophages play a pivotal role in clearance of Af conidia



Macrophage lysosomes are essential for iron recycling



CFTR depletion and iron overload lead to lysosomal leakage



Methods

We examined the tracheal iron levels before and after transplantation in the CFTR^{-/-} mouse and the control by immunofluorescence staining and a colorimetric assay. Murine CFTR^{-/-} mφ phagocytosis and killing of Af conidia was evaluated by flow cytometry and confocal microscopy. Iron-induced reactive oxygen species (ROS) were assessed by flow cytometry, and lysosomal leakage and loss by immunofluorescence staining and confocal microscopy. The depth of Af invasion in the OTT (CF vs control) was evaluated by Grocott methenamine silver staining. Finally, we examined airway biopsies from CF-LTRs for iron-laden mφs using Prussian blue stain and measured bronchoalveolar lavage (BAL) iron content using inductively-coupled mass spectrometry (ICP-MS)

Results

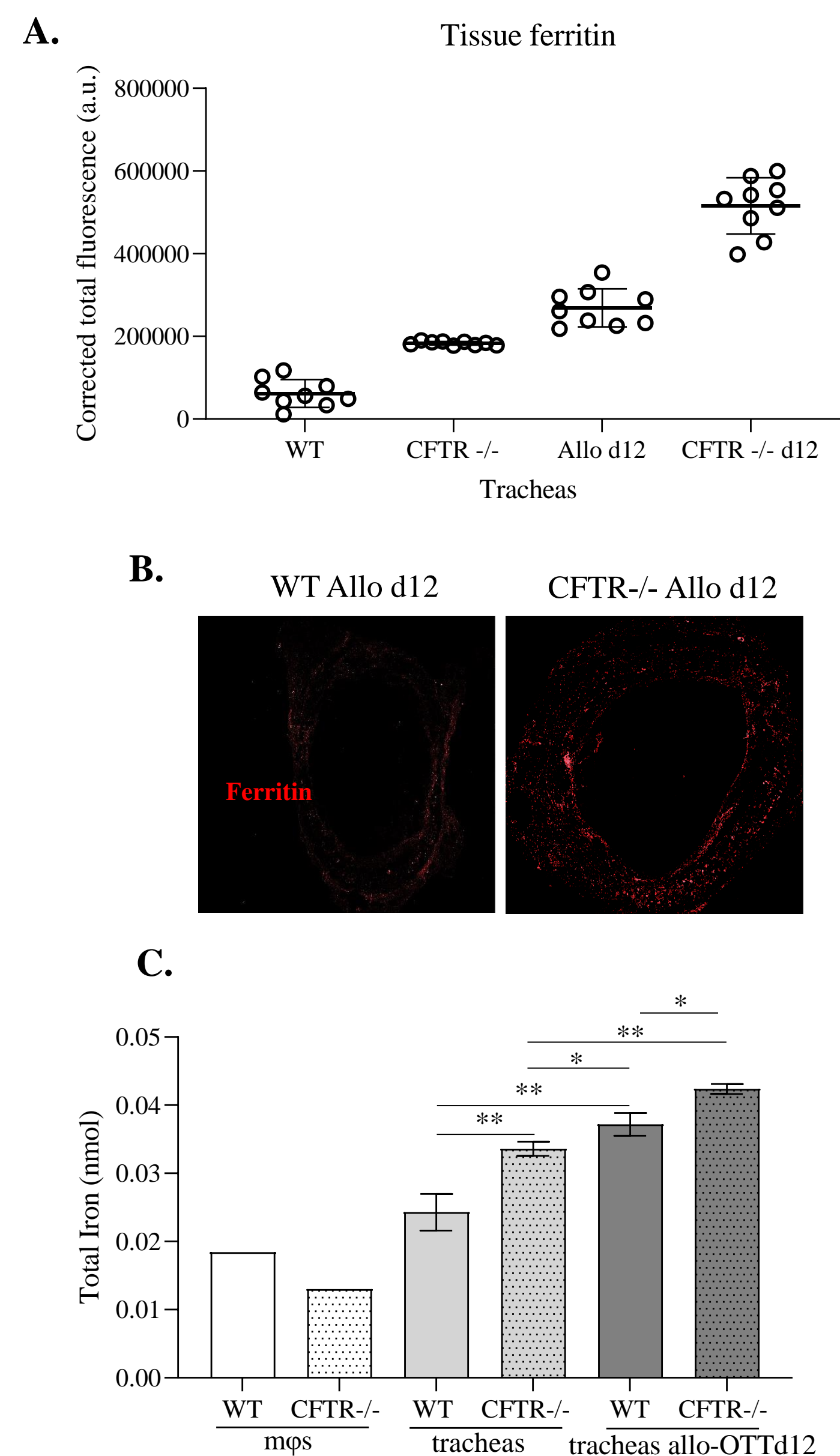


Figure 1. Iron levels are higher in the CFTR^{-/-} murine airway both at baseline and after transplant compared to the WT control. A. Quantification of tissue ferritin. B. Representative confocal microscopy images of tracheas stained for ferritin. C. Quantification of total tracheal iron measured by iron colorimetric assay.

Results

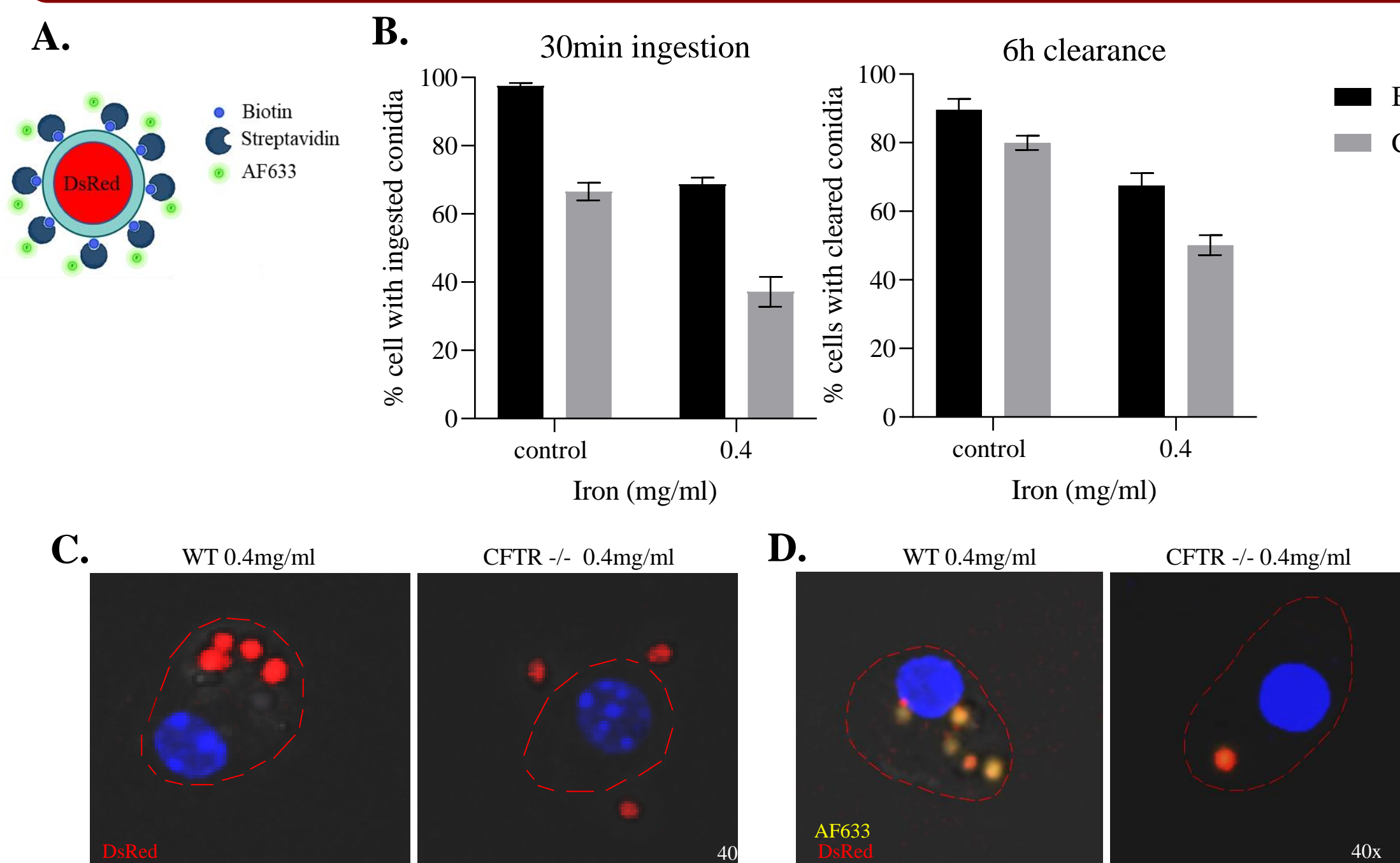


Figure 2. CFTR^{-/-} macrophages showed decreased ability to phagocytose and kill Af conidia. Iron addition further impairs phagocytosis and killing. A. DsRed expressing conidia labeled with AF633. B. Flow cytometry analysis of cells that ingested and cleared conidia. C. Representative confocal image of Af conidia phagocytosis. D. Representative confocal image of Af conidia killing.

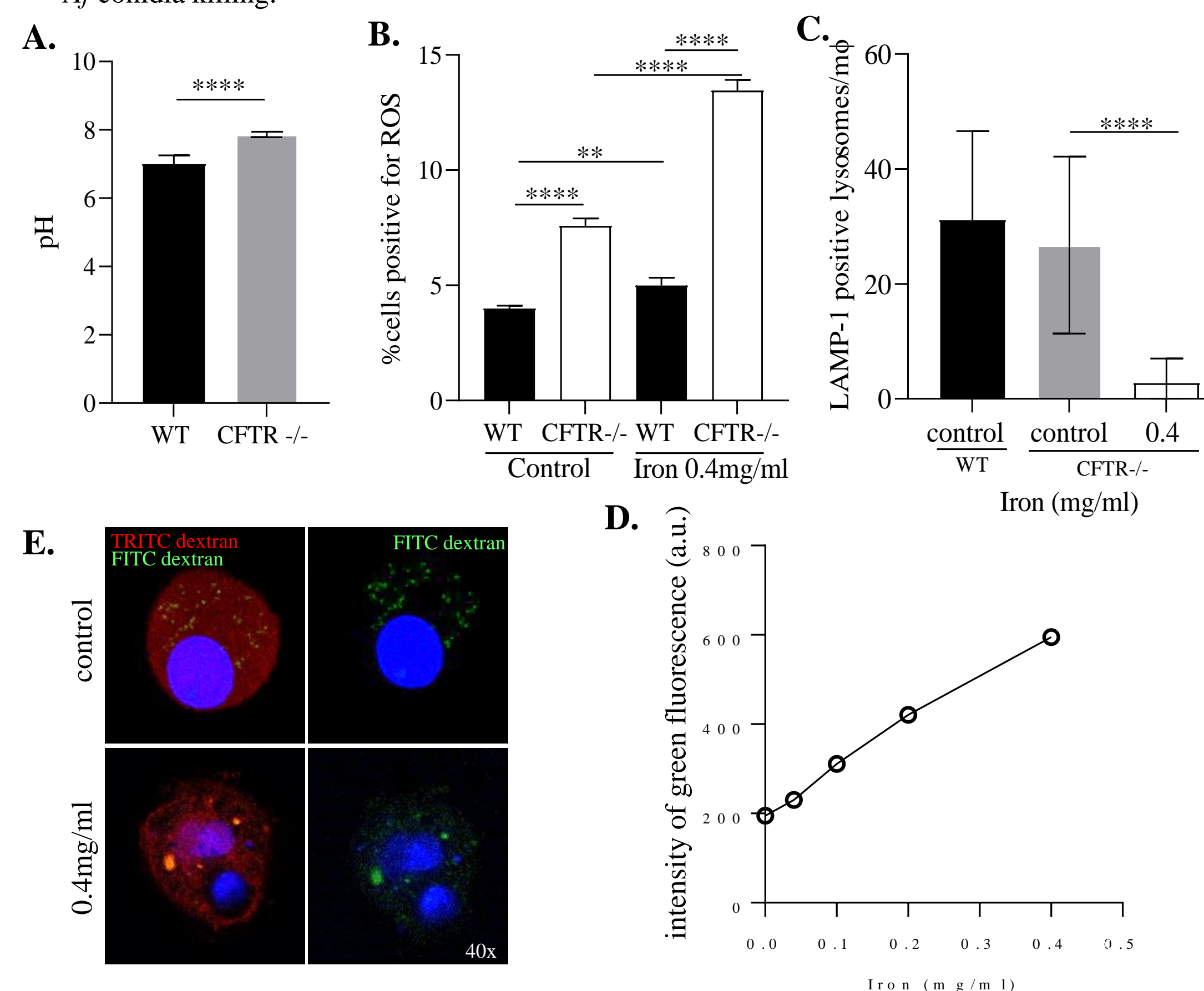


Figure 3. CFTR^{-/-} macrophages showed increased pH and increased ROS at baseline. Iron addition in CFTR^{-/-} macrophages caused lysosomal leakage and decrease of lysosomal number. A. pH measurement by LysoSensor™ Yellow/Blue dextran staining. B. Quantification of cellular ROS by DCFDA/H2DCFDA staining and flow cytometry analysis. C. Quantification of LAMP-1 positive stained lysosomes per macrophage. D. Representative image of lysosomal leakage measured by FITC-dextran/TRITC-dextran staining. E. Quantification FITC-green fluorescence to analyze lysosomal leakage.

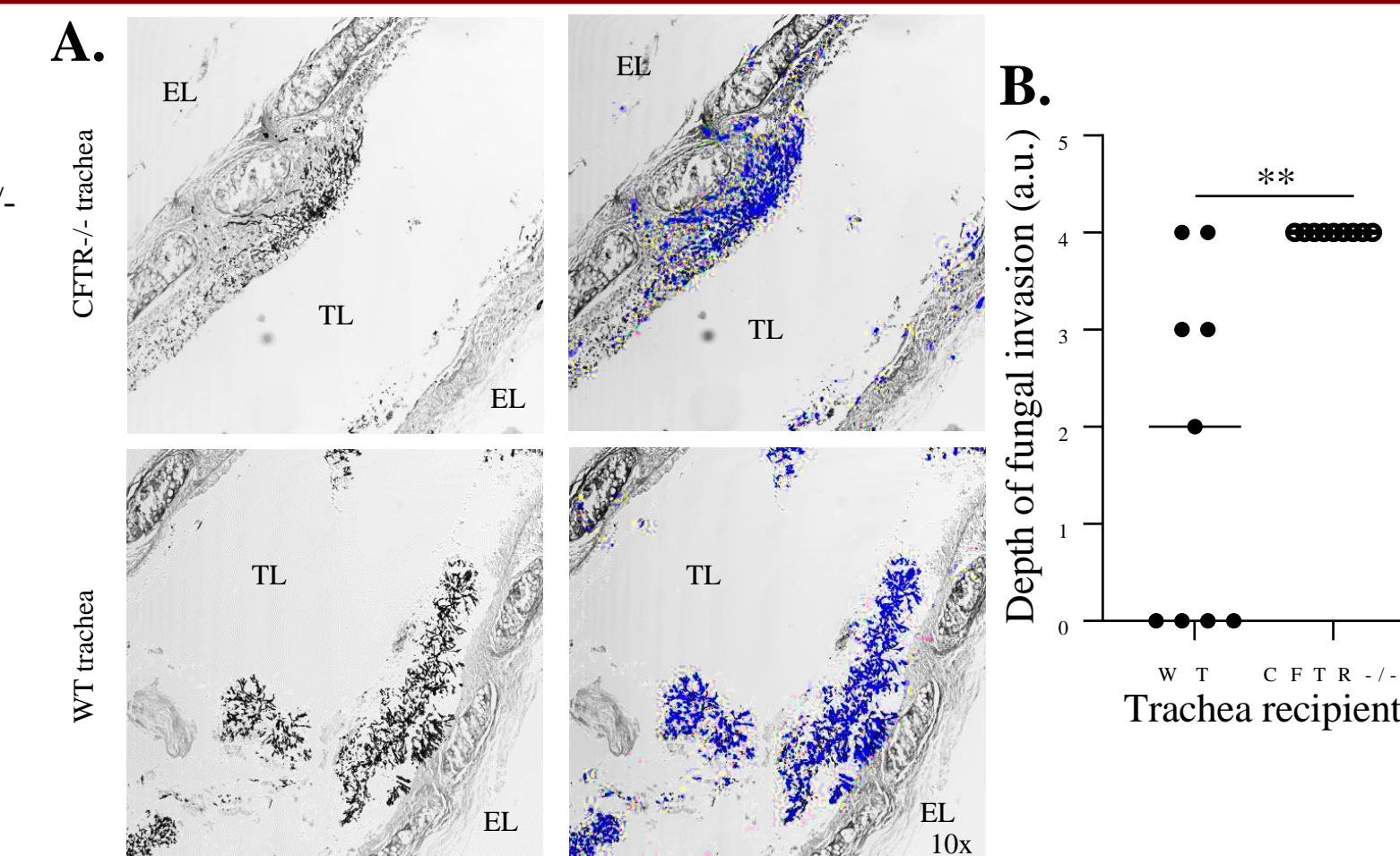


Figure 6. Aspergillus invasion in CFTR^{-/-} OTT compared to WT control. A. Representative confocal microscopy image of fungal invasion. B. Grading of fungal invasion. TL: tracheal lumen, EL: extra tracheal lumen.

Conclusions

The CFTR^{-/-} murine tracheas have higher baseline iron content compared to the non-CF tracheas. This was also observed in our clinical samples from LTRs.

- Murine CFTR^{-/-} mφs have a decreased ability to phagocytose and kill Af conidia. The of addition of iron further impairs CFTR^{-/-} mφ ability to kill Af conidia.
- CFTR^{-/-} macrophages have higher pH and ROS at baseline compared to WT control.
- Iron induces lysosomal leakage and reduces lysosomal numbers in CFTR^{-/-} mφs.
- Af invasion was uniformly the highest grade in the murine OTT model in CFTR^{-/-} recipients compared to control recipients.

These results suggest that the innate immune response in the CF LTR may be significantly impaired by high transplant iron levels, through increased mφ lysosomal leakage and loss, decreasing their ability to kill Af, a factor that may explain the higher rates of invasive aspergillosis in CF LTRs.

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Contact Information

E. I. Matthaïou: Division of Pulmonary Allergy and Critical Care Medicine, Stanford University Medical Center, 1701 Page Mill Road, Office 168.20, Palo Alto, CA 94304, USA
Tel.: +1 650 497 6766; e-mail: ematth@stanford.edu

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