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14. ABSTRACT

The overarching goal of this Program is to develop the scientific knowledge needed to predict and prevent the progression of IPF. We postulate that IPF is caused by recurrent injury/repair/regeneration at the bronchoalveolar junction secondary to overexpression of MUC5B, mucociliary dysfunction, retention of particles, ER stress, and disruption of normal reparative and regenerative mechanisms in the distal lung. During the first year of funding, we have (1) obtained local and DoD approvals for human and animal research; (2) enrolled 26 first degree relatives of individuals with IPF and completed all study procedures for Project 1; (3) performed ChIP, MNase, and TF binding assays to show that MUC5B promoter region is hyperchippable and that HIF1 and GCF bind in this region (Project 2); (4) imported and bred new strains of mice (St3gal3, Fut2, Ern2, Ift88, and Arl13b) in Projects 3 and 4; (5) developed and assessed the amounts and glycosylation of Muc5b in mouse models at baseline, and identified changes in polymer size and migration after inflammatory challenge (Project 3); (6) identified 10 weeks post-injury as a key timepoint for increased ciliogenesis in Muc5b Tg mice and began characterization of ciliogenesis in human lung, and (7) presented findings at two international conferences and published two manuscripts.

15. SUBJECT TERMS
preclinical pulmonary fibrosis, biomarkers, airway mucin, mucin 5b polymer, mucociliary dysfunction, transcriptional regulation, lung repair, lung regeneration, ER stress, ciliogenesis

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Annual Technical Progress Report
Reporting period: 9/30/18-9/29/19

Introduction
The overarching challenge of this Program is to develop the scientific knowledge needed to predict and prevent the progression of Idiopathic Pulmonary Fibrosis (IPF). IPF affects 5 million worldwide, disproportionately affects men, is associated with cigarette smoking and combat-related particulate exposures, increases with age, is inexplicably increasing in prevalence, is a source of morbidity and mortality among military personnel, and is likely underdiagnosed. Patients with IPF are usually diagnosed when the fibroproliferative process has caused permanent and extensive lung parenchymal damage. Given the irreversible nature of this disease, even approved treatments for IPF only modestly slow progression and have not been shown to alter the 3-5 year survival following diagnosis. We have found that: 1) a gain-of-function *MUC5B* promoter variant rs35705950 is the strongest risk factor (genetic and otherwise) for the development of IPF, accounting for at least 30% of the risk of disease; 2) rs35705950 can be used to identify individuals in the preclinical phase of this life-threatening disease; 3) MUC5B represents a key molecule to understand the mechanisms that initiate the fibroproliferative process in the bronchoalveolar epithelium; and 4) focusing on MUC5B may provide a unique opportunity to define the early molecular events that lead to the development of IPF. We propose that a comprehensive, multi-dimensional approach that focuses on MUC5B transcription in airway epithelia, biological consequences of MUC5B overproduction that are mediated by airway epithelia and cilia, and biomarkers to predict preclinical pulmonary fibrosis (PrePF) and identify those at risk of disease progression could conceivably change the approach in IPF from palliative to preemptive.

Keywords
Preclinical pulmonary fibrosis, biomarkers, airway mucin, mucin 5b polymer, mucociliary dysfunction, transcriptional regulation, lung repair, lung regeneration, ER stress, ciliogenesis

1. Accomplishments

a. What were the major goals of the project?
- *List the major goals of the project as stated in the approved SOW. If the application listed milestones/ target dates for important activities or phases of the project identify these dates and show actual completion dates or the percentage of completion.*

Project 1
Specific Aim 1: Screen 500 asymptomatic siblings of sporadic IPF cases and perform pulmonary function testing on cases of preclinical pulmonary fibrosis (PrePF).
- Subtask 1: Coordinate with the site PIs to obtain IRB approval at each site for this study.
- Milestone #1: Secure IRB approval at all sites for subject recruitment.
- Subtask 2: Coordinate with the Investigators to consent IPF subjects to contact their siblings for study recruitment.
- Milestone #2: Prepare and submit manuscript on the prevalence and radiographic features of PrePF in the siblings of patients with sporadic IPF.

Specific Aim 2: Develop and validate a biomarker profile that improves the detection of preclinical pulmonary fibrosis (PrePF).
- Milestone #3: Prepare manuscript on development of peripheral blood biomarker profile of PrePF.

Specific Aim 3: Elucidate the determinants of progression in preclinial pulmonary fibrosis (PrePF).
- Major Task 1: Recontact all subjects with Year 1-3 scans positive for PrePF and perform repeat HRCT 2-3 years after study enrollment.
- Major Task 2: Re-contact subjects with Year 1-3 scans positive for PrePF and perform repeat PFTs 2-3 years after initial study enrollment.
- Major Task 3: Recontact all subjects with initial Year 1-3 scans positive for PrePF and perform repeat peripheral blood draw.
- Milestone #4: Prepare manuscript on development of peripheral blood biomarker profile of progressive PrePF.

Project 2
Specific Aim 1: Elucidate Molecular regulation of MUC5B in relationship to the SNP and publish paper reporting on mechanism of regulation (months 1-12 research, 75% completed; months 12-18 for publication, 85% completed)
Specific Aim 2: Determine integrated transcriptional control of MUC5B expression in response to pro-fibrotic signals and publish manuscript reporting on integrated regulation of MUC5B expression (months 12-30 research, 35% completed; months 30-42 publication, 0% completed)
Specific Aim 3: Elucidate the impact of MUC5B variant on airway wound healing and proteostasis and publish manuscript on functional impact of MUC5B variant in airway epithelia (Months 30-48; 0% completed)

Project 3 **Specific Aim 1: Demonstrate that MUC5B/Muc5b overproduction by club cells and T2 cells in distal airways promotes dysfunctional MCC.**	**Timeline Years 1-2**	**Completion**
Major Task 1: Regulatory approval, establishment of mouse colonies.	Months	
Subtask 1: Regulatory approval of animal research.		100% (1/2018)
Milestone #1: Secure IACUC approval at University of Colorado AMC.	0	
Milestone #2: Secure ACURO approval.	0-3	
Subtask 2: Animal breeding for experiments.		#1: 100% (1/2018) #2 established by 1/2018, and will continue through full grant period
Milestone #1: Import C57BL/6J mice and ROSA$^{mT/mG}$ strains.	0-3	
Milestone #2: Breed C57BL/6J, Scgb1a1-Muc5b Tg, SFTPC-Muc5b Tg, Scgb1a1Cre$^{ER/+}$;Muc5b$^{lox/lox}$; SftpcCre$^{ERT2/+}$;Muc5b$^{lox/lox}$; ROSA$^{mT/mG}$ mice.	3-18	
Major Task 2: Demonstrate that Muc5b overproduction in murine PF impairs MCC and mucus transport *in vivo* and *in vitro*.	Months	
Milestone #1: Acute and Chronic MCC in Scgb1a1-Muc5b Tg, SFTPC-Muc5b Tg, Scgb1a1-Muc5b$^{\Delta/\Delta}$; SftpcCre$^{ERT2/+}$;Muc5b$^{\Delta/\Delta}$	0-12	#1: 75% #2: 50%
Milestone #2: Mucus transport in primary cultures of lung epithelia from Scgb1a1-Muc5b Tg, SFTPC-Muc5b Tg, Scgb1a1-Muc5b$^{\Delta/\Delta}$; SftpcCre$^{ERT2/+}$;Muc5b$^{\Delta/\Delta}$ and human cells ±IPF and ±rs35705950 'T' allele	0-12	
Milestone #3: Statistical analysis of Data	12-18	#3: 33%
Major Task 3: Demonstrate that aberrantly glycosylated MUC5B/Muc5b accumulates in the airways in PF.	Months	
Milestone #1: Quantify MUC5B/Muc5b, SCGB1A1/Scgb1a1, SPC, MAL II, and UEA I labels human and mouse lung tissues by histology.	6-18	#1-2: 100% complete (10/2019)
Milestone #2: Demonstrate colocalization of secreted MUC5B/Muc5b with glycan markers	6-18	
Milestone #3: Statistical analysis of Data	12-18	#3: 75% #4-5: 100% (10/2019)
Milestone #4: Manuscript preparation and submission: Data from Aim 1	9-12	
Milestone #5: Manuscript acceptance and publication: Data from Aim 1	12-18	
Specific Aim 2: Determine whether Muc5b-dependent pro-fibrotic effects in mice are induced by aberrant mucin biosynthesis and proteostasis programs in club cells and T2 cells	**Timeline Years 2-3**	
Major Task 1: Characterize the dependence of glycosylation of Muc5b on gene expression levels and cellular source.	Months	

Milestone #1: Demonstrate colocalization of intracellular MUC5B/Muc5b with glycan markers and cell specific markers.	13-24	#1-2: 75%
Milestone #2: Identify changes in polymer size and migration by Western.	13-24	
Milestone #3: Statistical analysis of Data	16-27	25%
Major Task 2: Determine the effects of Muc5b levels and localization on mucin biosynthetic enzyme expression in bleomycin-induced fibrosis.	Months	
Milestone #1: Isolated and purify cells from fluorescent-tagged mice	20-26	#1-2: 50%
Milestone #2: Analyze St3Gal1- St3Gal6, St6Gal1- St6Gal2, Fut1-Fut11, and Agr2 transcript and protein levels.	24-27	
Milestone #3: Statistical analysis of Data	27-30	
Major Task 3: Determine the effects of Muc5b levels and localization on proteostasis dysfunction in bleomycin-induced fibrosis.	Months	
Milestone #1: Identify significant UPR/ER stress markers Atf6, Ern1(IRE-1α), Ern2 (IRE-1β), Ddit3 (CHOP), Hspa5 (Grp78/BiP), Eif2ak3 (PERK), and Xbp1/spliced Xbp1 in Muc5b-overexpressing mice.	24-27	#1-2: 10%
Milestone #2: Confirm protein levels & localization of markers above.	27-30	
Milestone #3: Statistical analysis of Data	30-32	
Major Task 4: Effects of mucin biosynthesis and proteostasis regulators on Muc5b protein synthesis and pro-fibrotic mediator production.	Months	
Milestone #1: Test ER Stress activation in MUC5B-expressing lung epithelial cell lines (A549, NCI-H292, and LC-2/ad) and NHBE's.	24-36	#1: 10%
Milestone #2: Test significance of ER Stress activation using lentiviral overexpression and shRNA-mediated knockdown.	30-36	
Milestone #3: Statistical analysis of Data	24-36	
Milestone #4: Manuscript preparation and submission: Data from Aim 2	27-32	
Milestone #5: Manuscript acceptance and publication: Data from Aim 2	33-36	
Specific Aim 3: Determine the critical mechanisms required for MUC5B/Muc5b to promote pulmonary fibrosis.	**Timeline Years 2-4**	
Major Task 1: In vivo studies.	Months	
Milestone #1: Breed St3gal3, Fut2, Agr2, and Ern2 (IRE-1β) knockout mice for experiments. Obtain other candidates as needed.	13-44	#1: 10%
Milestone #2: Test effects genetic deficiency in in vivo *models above on Muc5b levels, localization, and glycosylation and on epithelial proteostasis, ER stress, and fibrosis.*	24-36	
Milestone #3: Test effects of pharmacologic and enzyme interventions in in vivo *models above on Muc5b levels, localization, and glycosylation and on epithelial proteostasis, ER stress, and fibrosis.*	33-44	
Major Task 2: In vitro studies.	Months	
Milestone #1: Test effects genetic deficiency in models above on mucus transport, and epithelial expression of pro-fibrotic mediators in vitro.	38-41	
Milestone #2: Test effects of pharmacologic and enzyme interventions on mucus transport, and expression of pro-fibrotic mediators in vitro.	40-46	
Major Task 2: Analysis and dissemination of Research.	Months	
Milestone #1: Statistical analysis of Data	13-48	
Milestone #2: Manuscript preparation and submission: Data from Aim 3 (two papers).	28-42	
Milestone #3: Manuscript acceptance and publication: Data from Aim 3 (two papers).	36-48	

Project 4
Aim 1: Determine the effect of Muc5b concentration on expression of cilium-associated genes in distal airway stem cell populations following injury in mice.
Major Task 1: Regulatory approval and animal breeding (scheduled for months 0-9; 100% complete).

Subtask 1: Regulatory approval of animal research.
Milestone #1: Secure IACUC approval at University of Colorado. Milestone set for 09-30-2017, completed 06-09-2017.
Milestone #2: Secure ACURO approval. Milestone set for 12-31-2017, completed 09-05-2017.
Subtask 2: Animal breeding for experiments.
Milestone #2: Breed enough Muc5b-/-, Scgb1a1-Muc5bTg and SPC-Muc5bTg for experiments to commence. Milestone set for 06-30-2018, completed 06-30-2018.

Major Task 2: Markers of ciliogenesis (Arl13b and Foxj1), Muc5b and Mmp7 will be co-localized with basal cell markers (Krt5, Krt14, and p63) and β-catenin following injury (scheduled for months 3-24; 100% complete).
Milestone #1: Treat Muc5b-/-, Scgb1a1-Muc5bTg and SPC-Muc5bTg mice with bleomycin and H1N1 virus. Collect tissue for IF staining. Milestone set for 09-30-2018, completed 09-30-2018.
Milestone #2: Perform IF staining, take images, and perform qualitative analysis of the image data. Milestone set for 03-31-19, completed 03-31-19.
Milestone #3: Perform quantitative analysis of the image data and statistical analysis. Milestone set for 09-30-2019, completed 09-30-2019.

Major Task 3: Identify changes in cilium gene expression in isolated DASC populations at multiple timepoints following injury (scheduled for months 3-18; 50% complete).
Milestone #1: Treat Muc5b-/-, Scgb1a1-Muc5bTg and SPC-Muc5bTg mice with bleomycin and H1N1 virus. Milestone set for 06-30-2018, completed 06-30-2019.
Milestone #2: Perform fresh lung tissue digests, DASC isolation, and RNA extractions. Milestone set for 09-30-2018, completed 09-30-2019.
Milestone #3: Run RT-qPCR on the Fluidigm platform. Milestone set for 12-31-2018, 25% completed.
Milestone #4: Statistical analysis of RT-qPCR data and prioritization of genes for Aim 2. Milestone set for 03-31-2019, 0% complete.

Major Task 4: Publication of findings from Aim 1 (scheduled for months 18-24; 75% complete)
Milestone #1: Prepare and submit manuscript. Milestone set for 09-30-2019, 75% completed.

Aim 2: Demonstrate that changes in cilium gene expression in airway progenitor cells affect injury/repair and fibrosis.
Major Task 1: Establish NHBE cell cultures, optimize lenti-shRNA and lenti-ORF protocols, and treatment concentrations (scheduled for months 0-18; 50% complete).
Milestone #1: Establish NHBE cultures, successfully inhibit and overexpress positive control genes. Milestone set for 06-30-2018, 100% completed.
Milestone #2. Optimize bleomycin and H1N1 virus concentrations. Milestone set for 03-31-2019, 75% completed.
Major Task 2: Inhibit and overexpress cilium genes, measure injury/repair, regeneration, and Wnt signaling (scheduled for months 18-36; 5% complete).
Milestone #1: Inhibit and overexpress cilium genes of interest. Milestone set for 12-31-2019, 20% completed.
Milestone #2: Treat cells in which cilium genes are inhibited/overexpressed with bleomycin and H1N1. Milestone set for 03-31-2020, 0% completed.
Milestone #3. Measure wound healing, TEER, Wnt signaling. Milestone set for 06-30-2020, 0% completed.
Milestone #4: Statistical analysis of the data and prioritization of genes for Aim 3. Milestone set for 09-30-2020, 0% completed.

Major Task 3: Determine the influence of cilium gene deletion on injury/repair, lung regeneration, and fibrosis in mice (scheduled for months 3-39; 25% complete).
Milestone #1: Breed Arl13 flox/flox and Ift8 flox/flox to Krt5-CreER mice. Breed CKO mice to Muc5b Tg or deficient lines. Treat with tamoxifen. Milestone set for 03-30-2019, 25% completed.
Milestone #2: Treat mice with bleomycin and H1N1. Collect tissue for analysis. Milestone set for 03-31-2020, 25% completed.
Milestone #3. IF staining for Arl13b, Foxj1, Muc5b, Mmp7 Krt5, Krt14, p63, and β-catenin. Milestone set for 09-30-2020, 25% completed.
Milestone #4. Measure collagen content of the lung by hydroxyproline and SHG assays. Milestone set for 09-30-2020, 25% completed.
Milestone #5: Statistical analysis of the data and prioritization of genes for Aim 3b. Milestone set for 12-31-2020, 0% completed.

Major Task 4: Publication of findings from Aim 2 (scheduled for months 36-42; 0% complete).
Milestone #1: Prepare and submit manuscript. Milestone set for 03-31-2021, 0% completed.

Aim 3: Determine the contribution of the MUC5B promoter variant on expression of cilium-associated genes in distal airway stem cell populations in IPF lung.
Major Task 1: Markers of ciliogenesis (ARL13B and FOXJ1), MUC5B and MMP7 will be co-localized with basal cell markers (KRT5, KRT14, and p63) and Wnt signaling marker β-catenin following injury (scheduled for months 0-36; 50% complete).
Milestone #1: Perform IF staining, take images, and perform qualitative analysis of the image data in IPF and control lungs. Milestone set for 03-31-2019, 100% completed.
Milestone #2: Perform quantitative analysis of the image data and statistical analysis. Milestone set for 09-30-2020, 50% completed.

Major Task 2: Measure expression of cilium genes identified in Aims 1-2 in DASCs from IPF and control lungs with and without Muc5b promoter variant (scheduled for months 0-42; 25% complete).
Milestone #1: Perform fresh lung tissue digests, DASC isolation, and RNA extractions from IPF and control lungs. Milestone set for 09-30-2020, 50% completed.
Milestone #2: Run RT-qPCR Taqman assays for genes from Aims 1-2. Milestone set for 12-31-2020, 25% completed.
Milestone #3: Statistical analysis of RT-qPCR data. Milestone set for 03-31-2021, 0% completed.

Major Task 3: Publication of findings from Aim 3 (scheduled for months 42-48; 0% complete).
Milestone #1: Prepare and submit manuscript. Milestone set for 09-30-2021, 0% completed.

b. What was accomplished under these goals?

- *For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.*

Project 1
Specific Aim 1:
• Recruitment of human participants is ongoing at open sites
• 142 first degree relatives of people with IPF referred for study participation
• 90 first degree relatives of people with IPF consented to study participation
• 40 first degree relatives of people with IPF have completed some, but not all study procedures
• 50 first degree relatives of people with IPF have completed all study procedures (informed consent, health questionnaire, blood draw, HRCT scan)
• Radiologic and clinical evaluation by thoracic radiologists and interstitial lung disease specialist clinicians of completed subjects is in process and ongoing
• No adverse events in the human subjects study
Specific Aim 3:
Follow up of preclinical cohort continues
• 313 subjects consented to follow up
• 73 subjects have completed some, but not all study procedures
• 237 subjects completed follow up (informed consent, health questionnaire, blood draw, HRCT scan)
• Radiologic and clinical evaluation by thoracic radiologists and interstitial lung disease specialist clinicians of completed subjects is in process and ongoing
• No adverse events in the human subjects study

Project 2
Major Activities:
1. Performed ChIP assays in multiple relevant cell types to define chromatin structure of the -3kB enhancer
2. Analyzed gene expression and chromatin structure in A549 cells with CRISPR-generated GG vs TT MUC5B enhancer genotypes
3. Performed ATAC-seq of primary airway epithelial cells derived from patients with IPF and normal

4. Performed PRO-seq in A549, LC2a/d and BEAS2B cells to determine sites of RNA polymerase 2 loading associated with active MUC5B transcription
5. Analyzed MUC5B enhancer fragment in reporter assays
6. Knocked down GCF and analyzed impact on MUC5B expression

SIGNIFICANT RESULTS/KEY OUTCOMES

We have made significant progress in understanding the molecular regulation of MUC5B in relationship to the variant and pro-fibrotic signals. We have used several assays of chromatin structure (Figs. 1 and 2) to establish that the region surround the IPF associated variant can be packaged in two different chromatin structures. One chromatin conformation is open and occurs in cells that express MUC5B, but also in lung cell types that fail to express MUC5B such as airway smooth muscle. A less accessible chromatin structure was found in THP1 cells, which also fail to express MUC5B (e.g. THP1 cells, Fig. 1C). These data suggest an epigenetic chromatin priming mechanism promotes aberrant MUC5B expression in IPF.

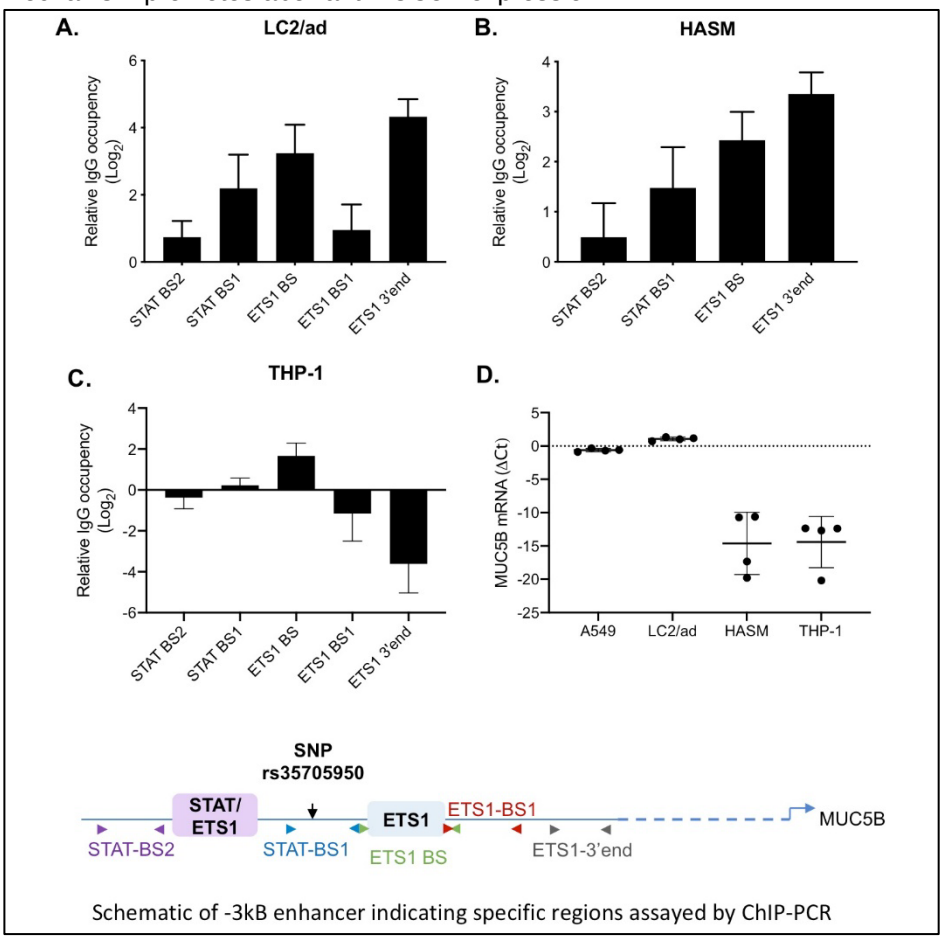


Figure 1. The *MUC5B* rs353705950 region is hyper-ChIPable in LC2/ad and airway smooth muscle cells but not in monocytes. **A-C.** IgG ChIP assay performed in cells as indicated shows that the region is hyper-ChIPable, which is strongly associated with open chromatin, in LC2/ad and human airway smooth muscle (HASM), but not in THP-1 cells. **D.** LC2/ad cells express MUC5B, whereas HASM and THP-1 cells do not express appreciable levels of MUC5B.

We confirmed the open chromatin structure at the -3kB MUC5B enhancer is relevant in primary cells using two techniques: ATAC-seq and also the IgG ChIP assay (Fig. 2).

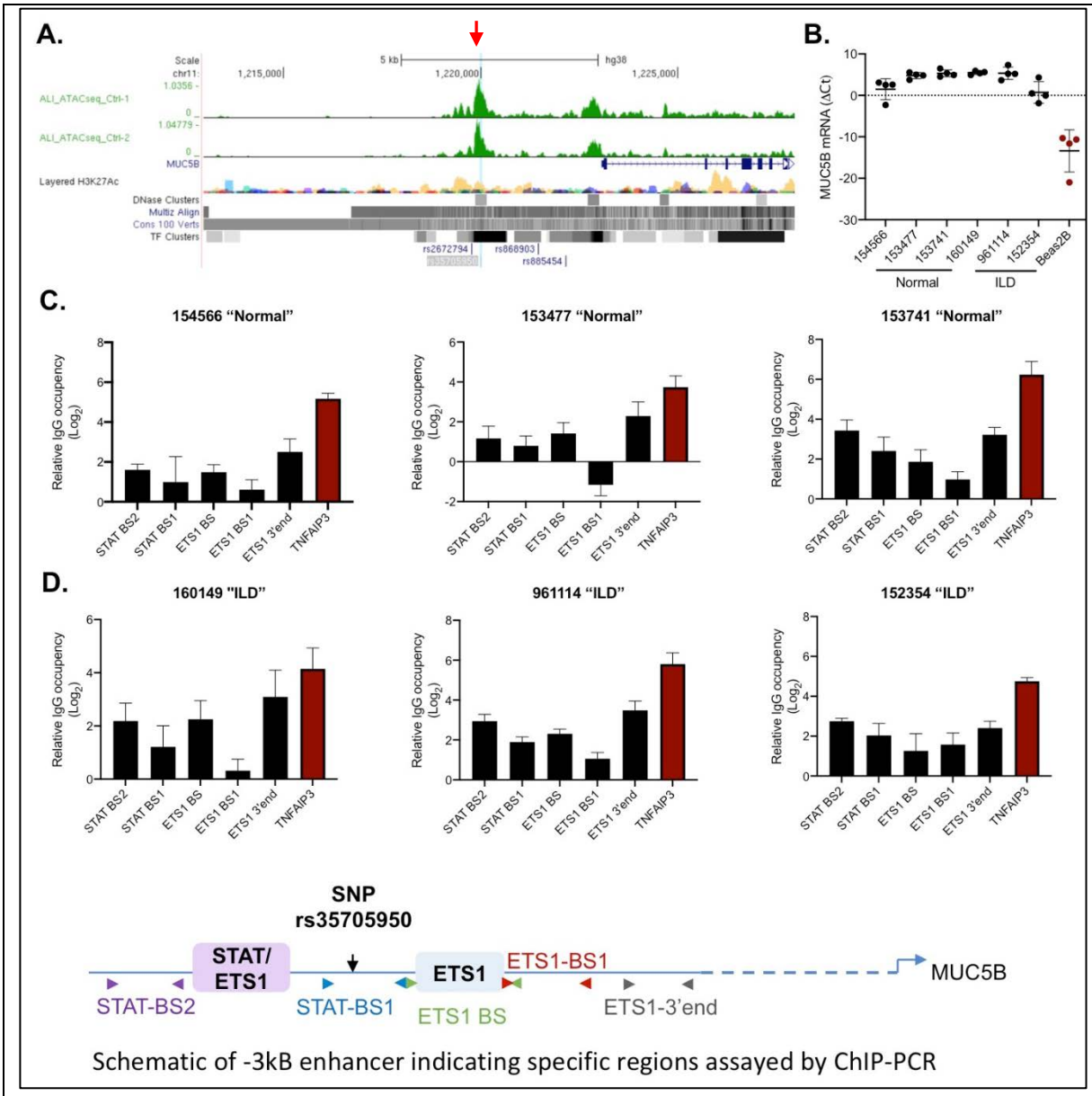


Figure 2. ATAC-seq and IgG Chip demonstrate an open chromatin structure for the MUC5B -3kB enhancer in primary human airway epithelial cells cultured at air-liquid interface. A. Assay for accessible transposase-sequencing (ATAC-seq) was performed on primary human airway epithelial cells cultured from a patient with ILD. Data for the MUC5B locus were visualized in the UCSC Genome Browser. A peak of transposase accessibility is clearly evident (red arrow) that aligns with the -3KB enhancer region and the IPF-associated variant. **B.** Cultured cells from subjects (control and ILD) express MUC5B. BeAS-2B cells, which do not expres MUC5B, are shown as c control. **C-D.** Primary cultures from 3 normal subjects and 3 patients with ILD (D) were assayed using IgG ChIP. The -3kB enhancer was "hyper-ChIPAble (i.e open chromatin) in all 6 subjects.

We have also used an approach called Precision Run-On sequencing (PRO-seq) to map RNA polymerase 2 activity at the MUC5B region. This high-resolution assay allowed us to determine that RNA polymerase 2 interacts directly with the -3kB enhancer and loads onto DNA within ~10 base pairs of the IPF-associated variant, strongly implicating the variant as having a direct role in enhancer function. The data show very clearly that the 3'UTR has a bidirectional (i.e. red and blue) PRO-seq signature, indicating RNA polymerase 2 loading. This implicates the 3 'UTR in the integrated control

of MUC5B expression (Aim 2 of this project)

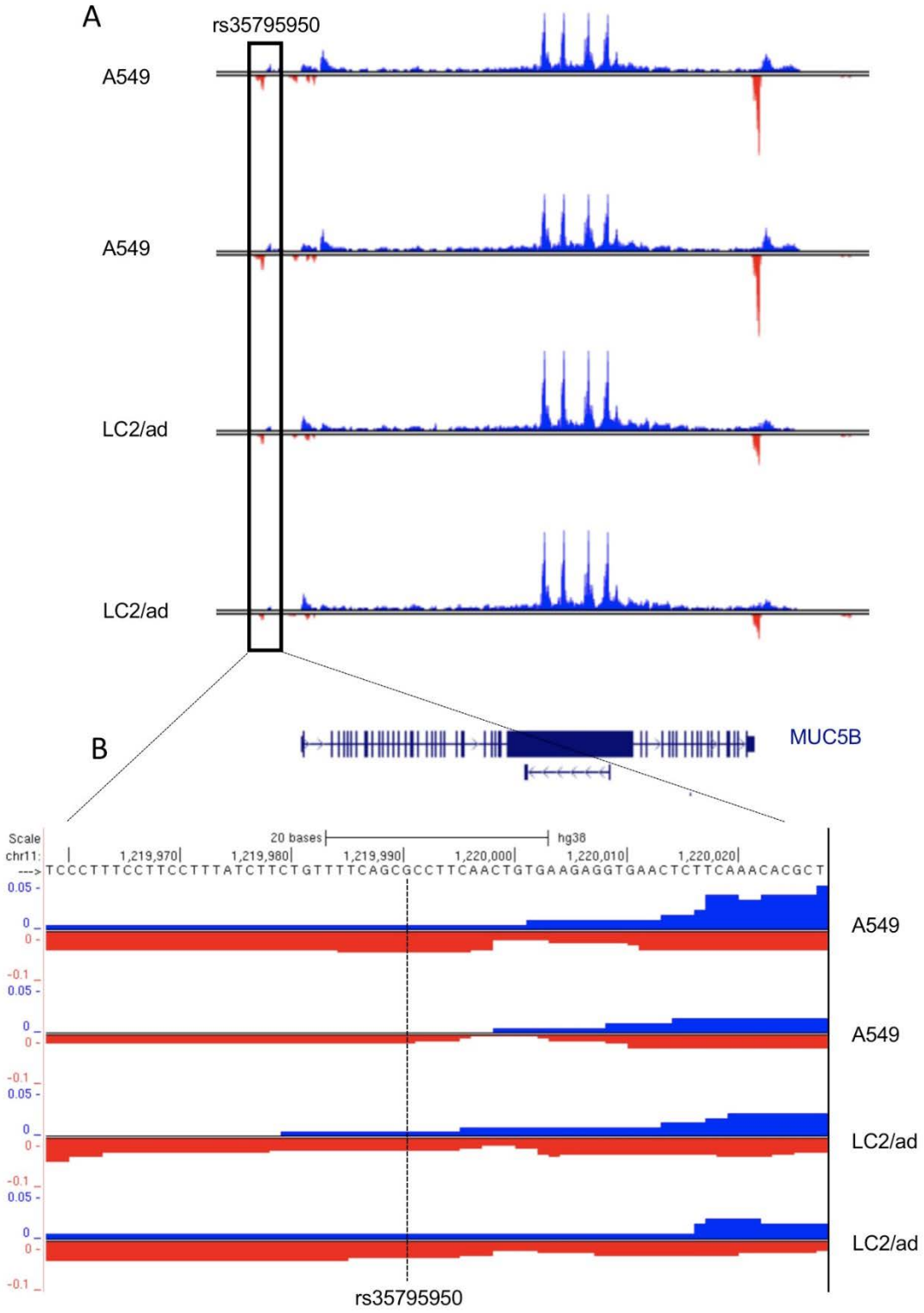


Figure 3. PRO-seq analysis of the MUC5B locus in A549 and LC2/ad cells reveals RNA polymerase 2 activity at the -3kB enhancer, the MUC5B gene body, and the 3'UTR. A. PRO-seq maps nascent RNA transcripts, a surrogate of RNA polymerase 2 activity. Bidirectional transcription, indicated by color of the transcript (i.e. red or blue), is known to reflect sites of RNA polymerase 2 loading on DNA. Here, the PRO-seq data for the entire MUC5B locus are visualized, showing bidirectional signatures at the -3kB enhancer, the start site, and the 3'UTR. **B.** Zoom in on the -3kB enhancer shows that RNA polymerase 2 loading occurs in close proximity to the site of the IPF-associated rs35795950 variant.

Project 3

Specific Aim 1: Demonstrate that MUC5B/Muc5b overproduction by club cells and T2 cells in distal airways promotes dysfunctional MCC.

Major Task 2: Demonstrate that Muc5b overproduction in murine PF impairs MCC and mucus transport in vivo and in vitro.

- We are conducting studies of acute and chronic MCC in mucin mutants in vivo an in primary epithelia from them in vitro.

Major Task 3: Demonstrate that aberrantly glycosylated MUC5B/Muc5b accumulates in the airways in PF.

- Milestone #1: Quantify MUC5B/Muc5b, SCGB1A1/Scgb1a1, SPC, MAL II, and UEA I labels human and mouse lung tissues by histology.
- Milestone #2: Demonstrate colocalization of secreted MUC5B/Muc5b with glycan markers
- Studies are underway presently, and as of 10/1/19 include 7 IPF GG, 7 GT and 5 IPF TT patient samples, as well as 6 GG and 5 GT controls. We have completed UEA-I and MAL-II lectin staining on all of the GG and GT samples, and we have completed statistical analysis on 15 of these for UEA-I and 10 of these for MAL-II. A batch of 10 MAL-II slides need to be re-labeled and analyzed.
- Four manuscripts published or in press.
    - ***Ann Am Thorac Soc.*** Mucociliary Defense: Emerging Cellular, Molecular, and Animal Models.2018. PMID: 30431350
    - ***Nature Communications***, Muc5b overexpression causes mucociliary dysfunction and enhances lung fibrosis in mice. 2018. PMID: 30560893
    - ***Am J Respir Crit Care Med***. Dawn of a New Era in the Diagnosis and Treatment of Airway Mucus Dysfunction. 2019. PMID: 30252497
    - ***JCI Insight***, Syndecan-1 promotes lung fibrosis by regulating epithelial reprogramming through extracellular vesicles. 2019. PMID: 31393853
    - ***Am J Respir Cell Mol Biol***. Muc5b Enhances Murine Honeycomb-like Cyst Formation. 2019. PMID: 31573335.

Specific Aim 2: Determine whether Muc5b-dependent pro-fibrotic effects in mice are induced by aberrant mucin biosynthesis and proteostasis programs in club cells and T2 cells.

Major Task 1: Characterize the dependence of glycosylation of Muc5b on gene expression levels and cellular source.

- We identified changes in polymer size, migration, and glycosylation by Western in mice at different times post bleomycin. MAL-II and UEA-I are completed in chronic models, and are being re-tested in single challenge bleomycin models.

Major Task 2: Determine the effects of Muc5b levels and localization on mucin biosynthetic enzyme expression in bleomycin-induced fibrosis.

- We deployed novel isolation and purification methods for lung epithelia using endogenous gene and transgenic reporters.
- We performed RNA-seq on cells isolated from transgenic mice, and we are using deep-sequencing to illustrate proteostasis pathways further.

Specific Aim 3: Determine the critical mechanisms required for MUC5B/Muc5b to promote pulmonary fibrosis.

Major Task 1: In vivo studies.

- We obtained Ern2 (IRE-1β) knockout mice from an outside source and we generated our own line on a C57BL/6 background
- We generated a new conditional St3gal3 knockout line
- We generated a new Muc5ac/Muc5b double knockout line.
- We are breeding St3gal3, Fut2, and Ern2 knockout mice for experiments.
- We have begun challenging St3gal3 and Fut2 lines with bleomycin.

Project 4:

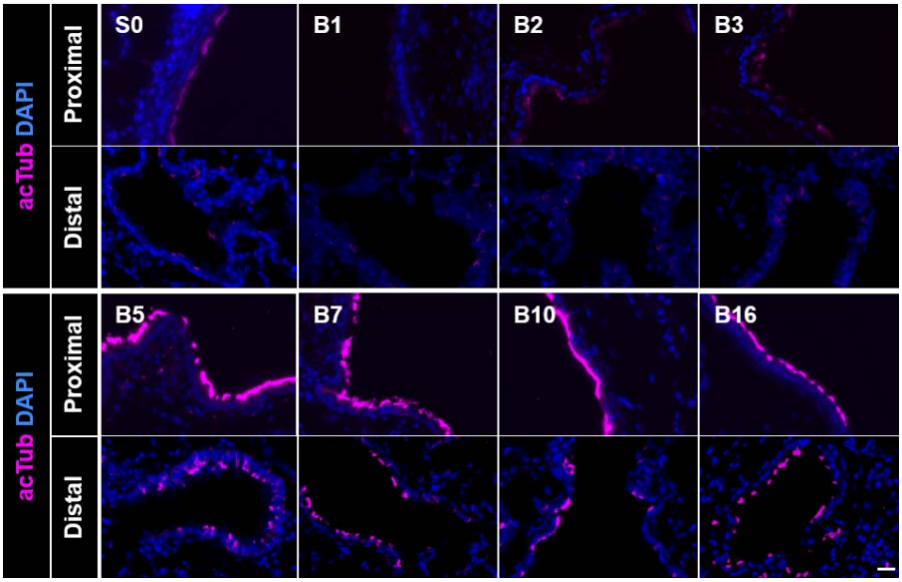
Aim 1: Determine the effect of Muc5b concentration on expression of cilium-associated genes in distal airway stem cell populations following injury in mice.

Major Task 1: Regulatory approval and animal breeding (scheduled for months 0-9; 100% complete).
We obtained IACUC approval at the University of Colorado 06-09-2017 and ACURO approval 09-05-2017 prior to start of funding and therefore ahead of the proposed milestone. Muc5b strain breeding commenced immediately after funding started and we have been able to breed sufficient numbers of animals to stay on track with experiments proposed in Aim 1. This task is completed.

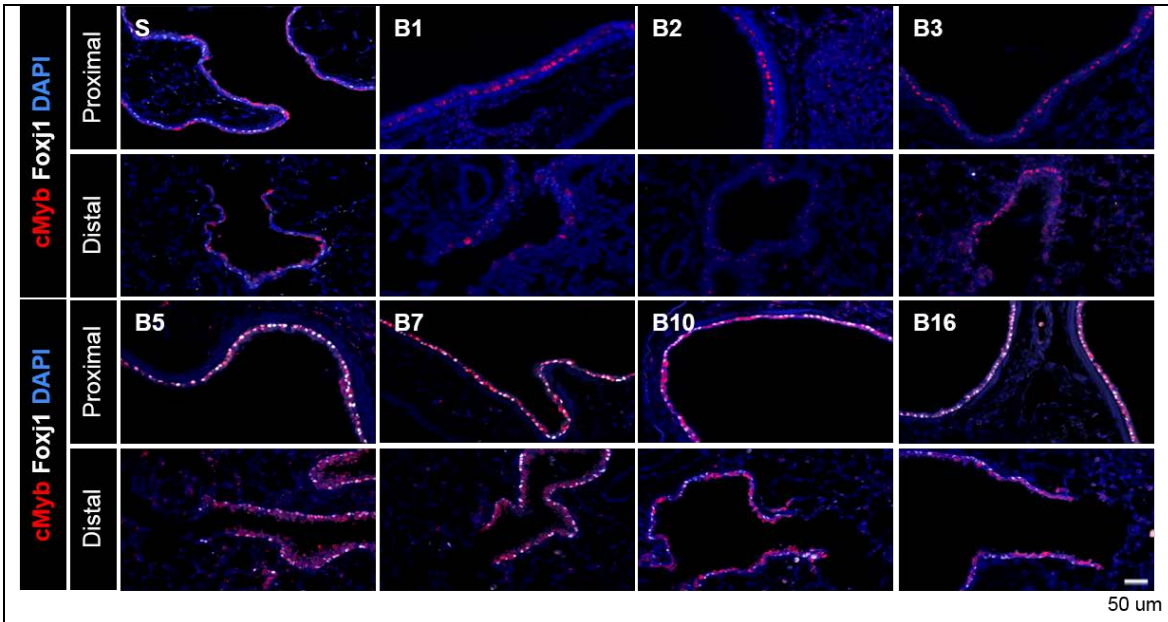
Major Task 2: Markers of ciliogenesis (Arl13b and Foxj1), Muc5b and Mmp7 will be co-localized with basal cell markers (Krt5, Krt14, and p63) and β-catenin following injury (scheduled for months 3-24; 100% complete).
We have collected tissue from Scgb1a1-Muc5bTg, SPC-Muc5bTg, wild type, and Muc5b-deficient mice using three animal models:

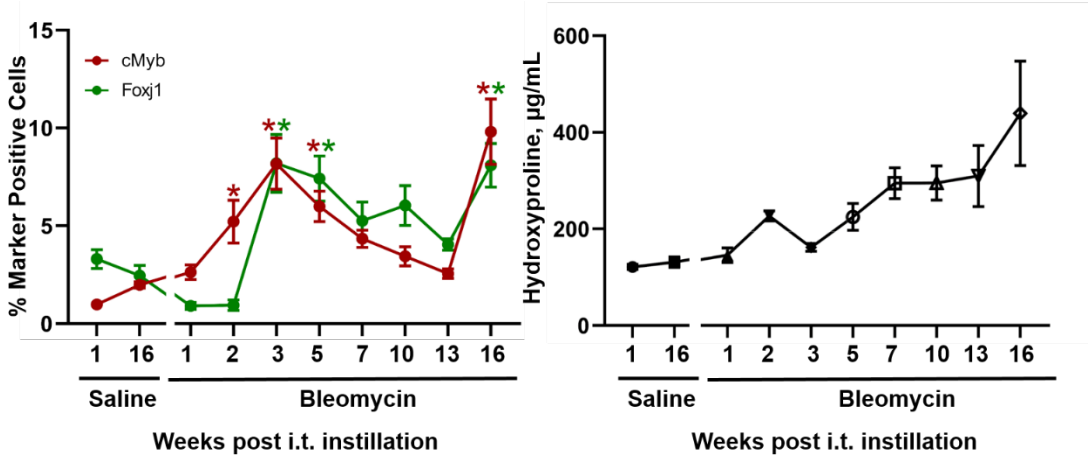
1. Single i.t. 2.5U/kg bleomycin on week 0; tissue collection at 1, 2, 3, 5, 7, 10, 13, 16 weeks post-bleomycin and 1 and 6 weeks post-saline for controls
2. Repeat i.t. bleomycin: 2.5 U/kg on week 0, 1.5 U/kg week 2 and week 4; tissue collection week 7 and 10
3. Single dose of H1N1/Puerto Rico/8/34 virus (ATCC VR-95) or saline i.n. for 1, 2, 3, or 16-20 weeks.

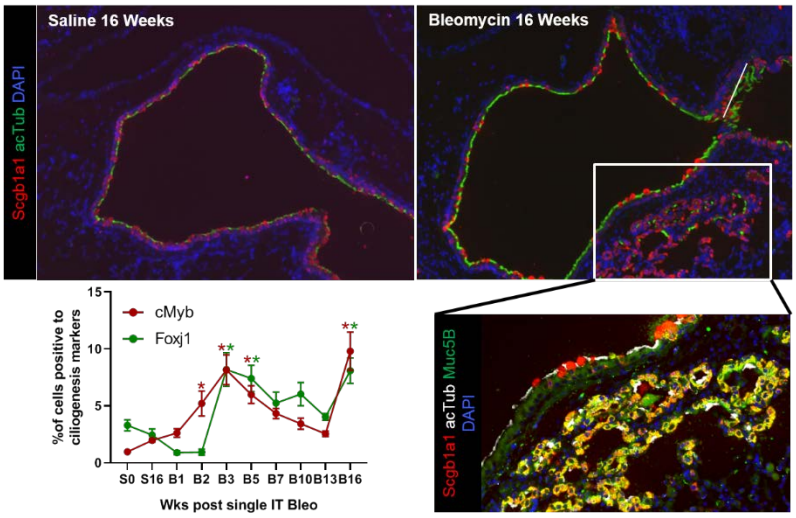
To characterize multiciliogenesis in the airways, wild-type mice were challenged intratracheally with a single dose of bleomycin and bleomycin-induced multiciliogenesis and fibrosis were assessed on week 1, 2, 3, 5, 7, 10, 13, 16 following IT bleomycin (saline 0 and 16 wks were used as controls). First, to confirm dynamic changes of multiciliated cells in response to IT bleomycin, we stained acetylated tubulin, a cilia axonome-specific protein. This confirmed the expected presence of acetylated tubulin positive motile cilia in saline controls. Remarkably, there was a decrease in staining of motile cilia in the airways and large areas of deciliated airways epithelium were evident at 1~3 wks. Fully recovered multiciliated cells are first observed at 5 wks post injury and surprisingly, intensity of motile cilia staining was highly increased persisting up to 16 weeks, compared to control. We are in the process of examining cilia by transmission electron microscopy (TEM) to examine the structure, lengths and numbers of cilia on multiciliated cells.



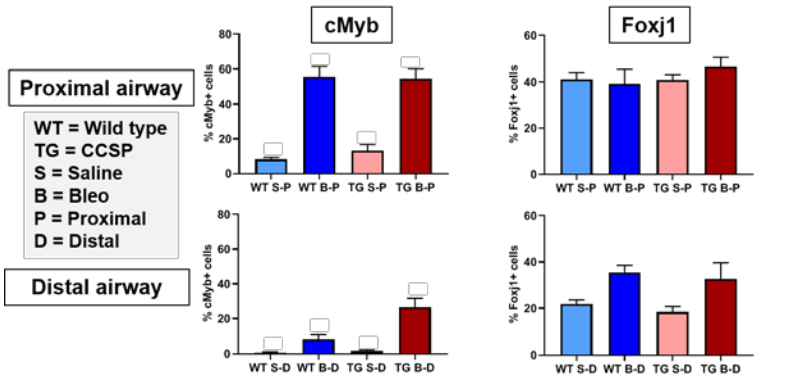
We next assessed the extent of multiciliogenesis by quantifying % of cMyb (marker of multiciliating cells) and Foxj1 (marker of fully multiciliated cells) by immunofluorescence (IF). cMyb showed high sensitivity to IT bleomycin as evidenced by the faster increase in cMyb positive cells at 1 wk reaching over 60% of airway cells, with significant differences as compared to saline control. It was surprising that the high levels of cMyb positive cells in the airways persist at all timepoints after IT bleomycin. The overall extent of labeling of Foxj1+ cells throughout the airways before injury in these experiments was 40%, however it was significant decrease to less than 2% at 1~3 wks. At 5 wks, however, a significant recovery in Foxj1+ cells was evident. Interestingly Foxj1+ cells also persisted at high levels compared to saline control up to 16 wks. Interestingly, IT bleomycin-induced fibrosis also persist at 5~16 wks indicating positive correlation of multiciliogenesis with fibrosis.



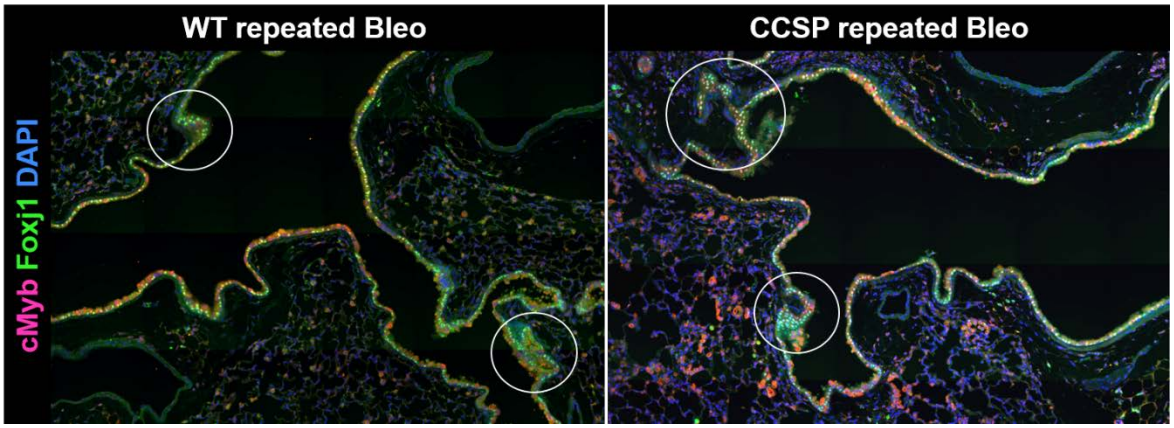


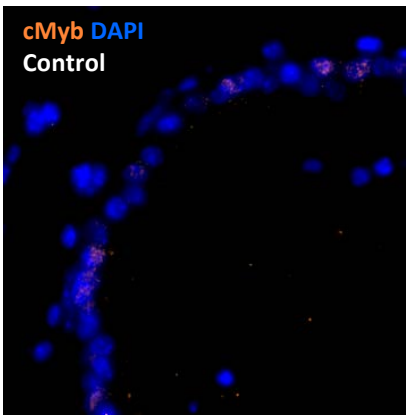


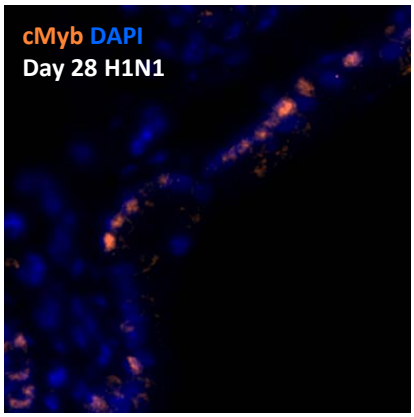
Consistent with IF data, flow cytometry data demonstrated slight, but significant changes both in Foxj1 and cMyb upon IT bleomycin during first 2~5 wks. Of note, % of cMyb and Foxj1+ cells at 7~13 wks are not statistically different from saline control probably because airways cells are diluted by type II cells as all isolated EpCam+ cells were used. It was interesting that decreased % of cMyb and Foxj1+ cells bounced back to the highest point at 16 wks. We confirmed that these changes observed by flow cytometry are due to multiciliogenesis both in airways and parenchymal region. It shows that prominent multiciliated cells in the airways and in the honeycomb cyst-like structure with MUC5B expression suggesting that multiciliogenesis is a global response occurring both in airway and parenchymal region.

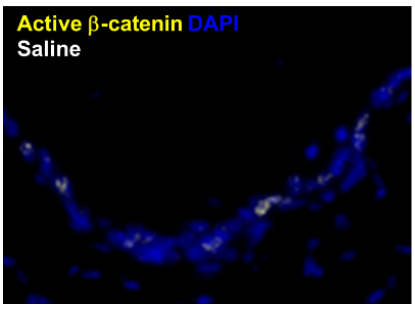


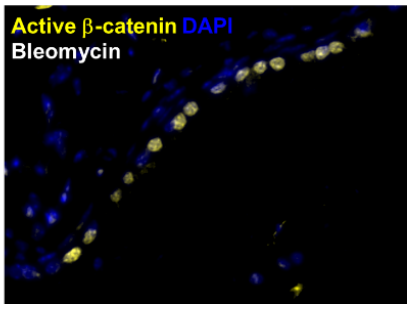
To determine the effect of excess Muc5B on multiciliogenesis, we challenged Scgb1a1-Muc5bTg and Tg-negative littermate control mice with three doses of IT bleomycin (2.5 U/kg, 1.5 U/kg, and 1.5 U/kg) over 10 weeks to strongly simulate the temporal heterogeneity and progressive nature of human IPF, as in our previous publication. Following repeated bleomycin challenge,











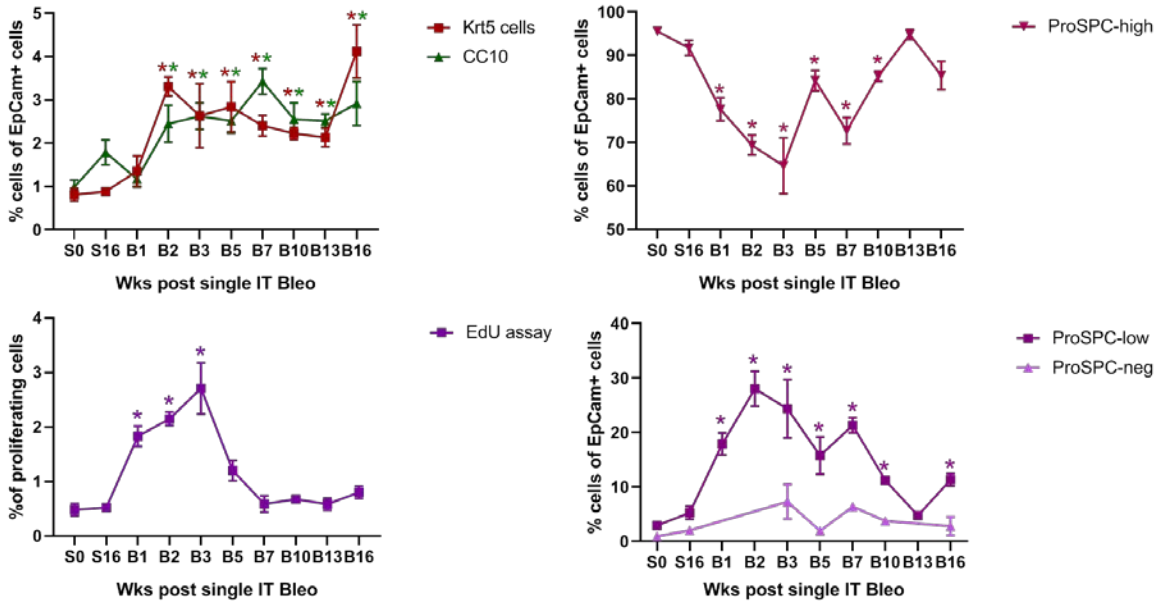
Scgb1a1-Muc5bTg mice showed a significant increase in % cMyb compared to Tg-negative littermates only in distal airways but no changes were found in proximal airways. For Foxj1, no significant changes were found in either proximal nor distal airways. Of note, Scgb1a1-Muc5bTg mice showed more cMyb+ and/or Foxj1+ pods, compared to Tg-negative littermates, in parenchymal regions. We also quantified collagen using confocal/multiphoton-excitation fluorescence microscopy with second harmonic generation (SHG). Quantification of SHG images demonstrates that Scgb1a1-Muc5bTg mice have significantly more collagen than Tg-negative littermate controls following challenge with bleomycin, as previously published by our group

In the H1N1 model, we have demonstrated active multicilogenes (by increased Myb staining) at 28 days post H1N1 exposure. In both models (bleomycin and H1N1), we have observed increased localization of active β-catenin to the nucleus (H1N1 data not shown).

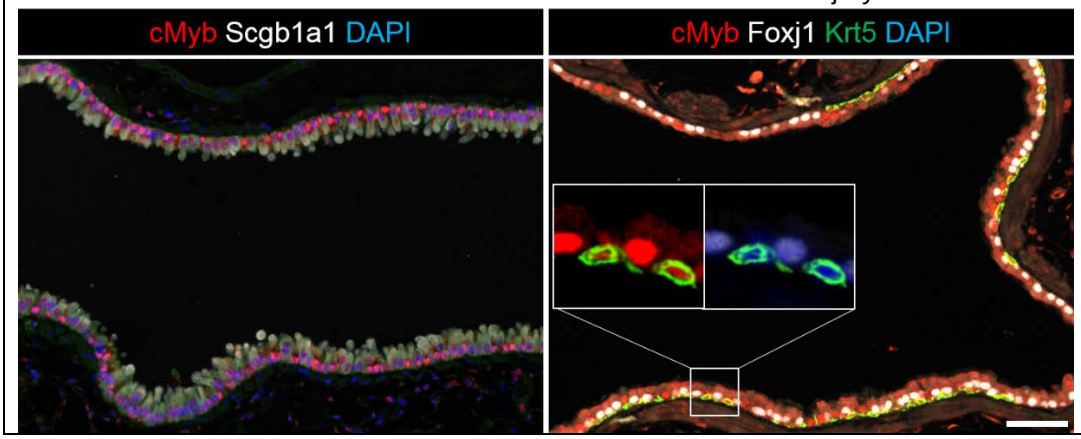
Major Task 3: Identify changes in cilium gene expression in isolated DASC populations at multiple timepoints following injury (scheduled for months 3-18; 50% complete).

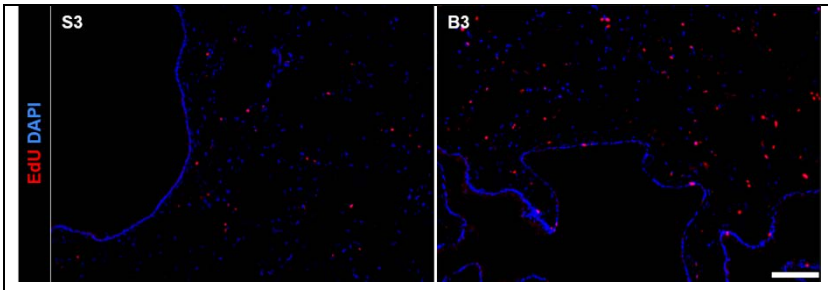
In light of the dynamic changes of multiciliogenesis post-blomycin, we examined how progenitor cells for multiciliated cells respond to i.t. bleomycin. Keratin 5 (Krt5) cell are believed to arise from immature stem/progenitor cells that are named distal airway stem cells (DASCs) or lineage-negative epithelial progenitors and these cells proliferate to form clusters of cells in the damaged alveolar regions. They

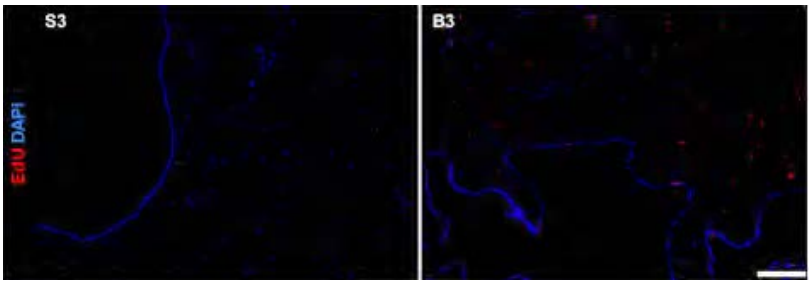
act as progenitors for both club and multiciliated cells. Club cells are also known to give rise to ciliated cells, as well as alveolar type I and II cells following injury. We confirmed that both Krt5 basal cells and club cells are increased when multiciliogenesis in the airways is induced as expected, whereas type II cells are decreased probably because of damage-induced apoptosis in alveolar region.



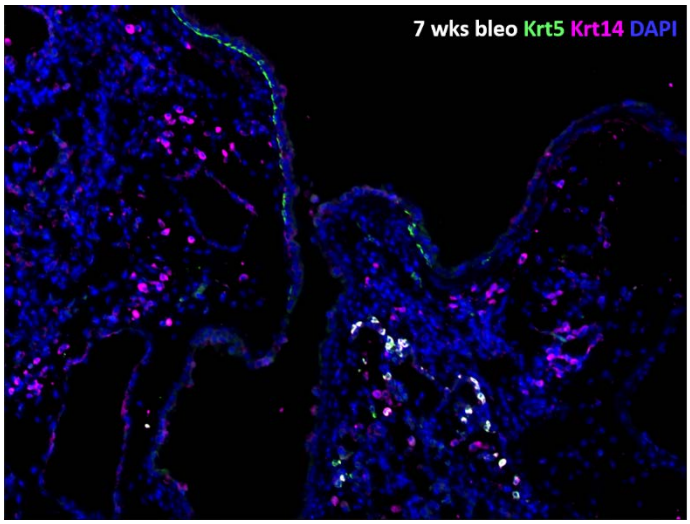
Next, we co-stained cMyb with Krt5 and Scgb1a1 to determine the progenitor cell type responsible for increased number of multiciliated cells. In accordance with previous publications, only a few Krt5 cells are detected in the airways in control mice, however, its expression is induced by IT bleo most prominently in proximal airways. These basal cells also express cMyb in the nucleus but not Foxj1 indicating initiation of multiciliogenesis. Club cells also express cMyb in the airways indicating that both Krt5 cells and club cells give rise to multiciliated cells following IT bleomycin but in different types of airways. These data indicate that both basal stem cells and club cells contribute to an increase in multiciliated cell numbers after the injury with bleomycin.







To confirm that ciliated cells were differentiated from Krt5 and club cells without undergoing cell division, we labeled proliferating cells using the EdU assay. Mice were injected with EdU 24 hr before harvesting. Proliferating cells were at their peak at 2 wks but only in alveolar regions. To confirm this lack of proliferation in the airways at 1 and 3 wks, we labeled proliferating cells with EdU for1 wk and showed that proliferating cells in the airways were barely detectable. Indeed, Krt5, Krt14 and Krt8 cells were induced only in the parenchymal region indicating that differentiation (remodeling) is prominent in airways and regeneration is prominent in p parenchymal region.

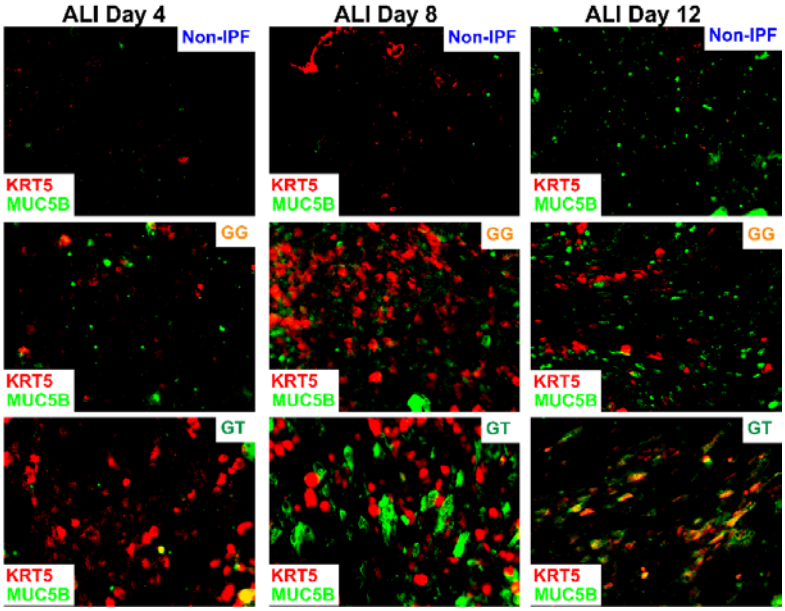


Given the small numbers and difficulty of isolating pure Krt5 cell populations, we are in the process of breeding enough mice to isolate Krt5 positive cells using the mTmG tag in Krt5-CreERT2-RosamT/mG; upon treatment with tamoxifen, Krt5 cells will be labeled with GFP and we can use flow cytometry to sort them using GFP. While waiting for these mice, we performed bulk RNA-seq on the sorted EpCam+ cells from SPC-Muc5bTg and Tg- littermates treated with a single dose of i.t. bleomycin. This analysis revealed a number of significant changes in gene expression at 1 week post-bleomycin, focusing mainly on genes involved in ER stress but also some ciliogenesis genes (Dync1h1). Much more pronounced changes were observed at 10 weeks post-bleomycin, including changes in ER stress, Notch signaling, Wnt/β-catenin signaling, and TGFβ signaling. Based on these bulk data, we have collected single cell profiles (scRNA-seq) of SPC-Muc5bTg and Tg- littermates at baseline and at 10 weeks post-bleomycin. We are in the process of analyzing both the baseline data (2 Tg+ and 2Tg- animals) and 10 week bleomycin (4Tg+ and 4Tg- animals) single cell datasets.

Major Task 4: Publication of findings from Aim 1 (scheduled for months 18-24; 75% complete)
Milestone #1: Prepare and submit manuscript. Milestone set for 09-30-2019, 75% complete.
We have prepared a manuscript describing our results so far; we are currently revising it and adding a few more experiments, and plan on submitting it before the end of 2019.

Aim 2: Demonstrate that changes in cilium gene expression in airway progenitor cells affect injury/repair and fibrosis.
Major Task 1: Establish NHBE cell cultures, optimize lenti-shRNA and lenti-ORF protocols, and treatment concentrations (scheduled for months 0-18; 50% complete).
We have been culturing NHBE cells, have optimized bleomycin concentration (to observe an increase in MUC5B protein secretion with no cell death; FC=fold change in the table below), and are in the process of optimizing H1N1 concentration. We have successfully inhibited miR-34 (25-100 fold

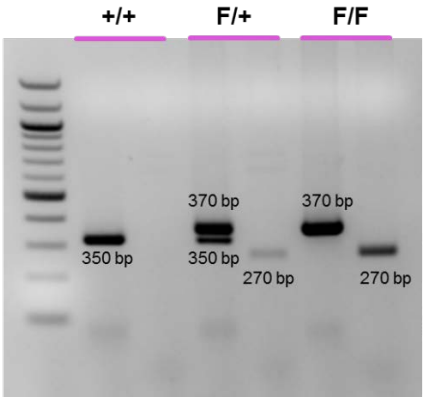
downregulation, depending on the concentration of the anatgomiR), a key micro RNA involved in multiciliogenesis and are in the process of analyzing cells from these cultures. We are in the process of analyzing gene expression profiles of treated cells.

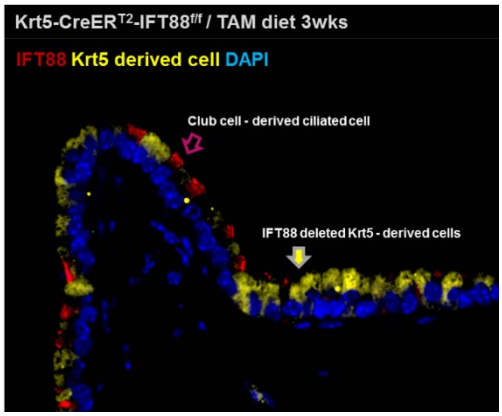


	secreted MUC5B protein			
	24hr FC	24hr p val	72hr FC	72hr p val
untreated	1.34	0.3109	1.73	0.1771
Bleo	1.73	0.2072	3.53	0.0439
Pam3Cys	2.03	0.1635	1.91	0.0721
poly(I:C)	2.05	0.0521	3.72	0.0507
LPS	2.89	0.1880	2.23	0.0776
TGFb	2.45	0.0560	2.52	0.1514

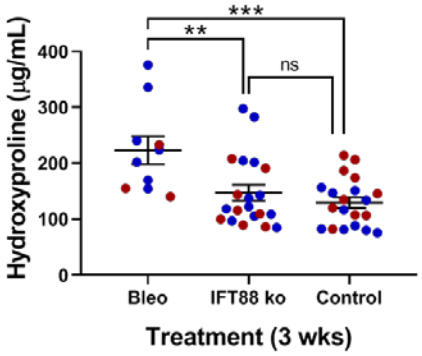
Major Task 3: Determine the influence of cilium gene deletion on injury/repair, lung regeneration, and fibrosis in mice (scheduled for months 3-39; 25% complete).

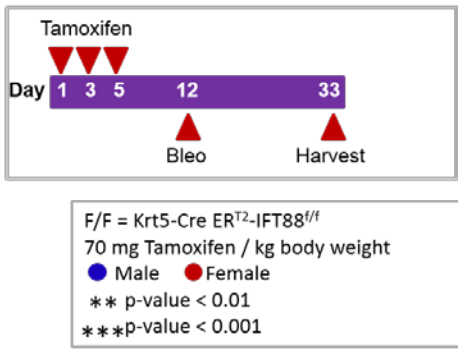
To assess the effect of cilium gene deletion in primary cilia on lung fibrosis in the bleomycin model, we conditionally deleted Ift88 in Krt5 cell using Krt5-CreERT2-IFT88$^{flox/flox}$ transgenic mouse. i.p. injection of 100 mg/kg tamoxifen for 4 days results in sufficient recombination, as monitored by allele-specific primers. Immunofluorescence demonstrates presence of Club cell-derived ciliated cells, suggesting that we will need to also breed Ift88 flox/flox and Scgb1a1-CreER mice to address the contribution of these club cell progenitors; this breeding is in progress.



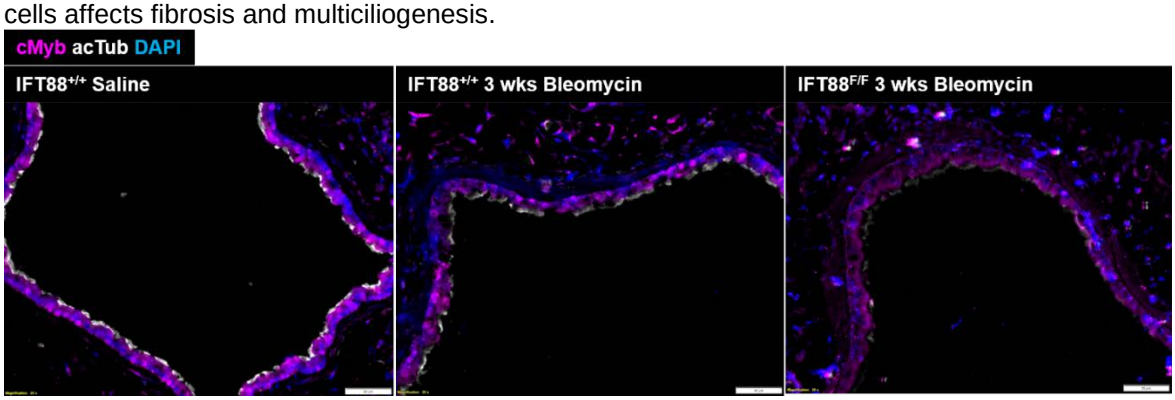


Lung fibrosis and multiciliogenesis in Krt5-CreERT2-IFT88$^{flox/flox}$ and controls were assessed at 3 weeks post single IT bleomycin. To determine whether Ift88 deletion in Krt5 cells reduces lung fibrosis, we assessed lung fibrosis biochemically by measuring HP content and the results demonstrated that Krt5-CreERT2-IFT88F/F mice have significantly less fibrosis than Krt5-CreERT2-IFT88+/+ mice following challenge with bleo. Next, we assessed multiciliogenesis in Krt5-CreERT2-IFT88F/F mice to determine whether multiciliogenesis is reduced by Ift88 deletion. While we expected a defect in primary cilia caused by Ift88 deletion to reduce mulitciliogenesis, the extent of the reduction ofcMyb following tamoxifen and IT bleo in Krt5-CreERT2-IFT88F/F mice down to almost undetectable was





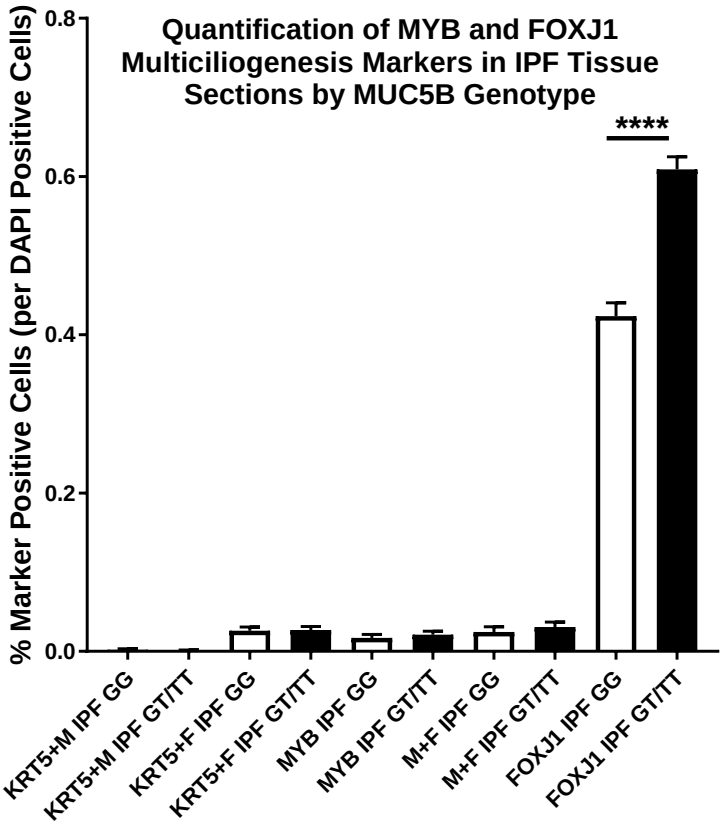
surprising. On the other hand, Krt5-CreERT2-IFT88+/+ mice showed induced cMyb in the airways as expected. These results indicate that deletion of a key cilium gene in primary cilia in Krt5 progenitor cells affects fibrosis and multiciliogenesis.



Arl13b flox/flox breeder mice were imported from Emory University and Mice will be ready by the end of October. This task is on track to be completed on time.

Aim 3: Determine the contribution of the MUC5B promoter variant on expression of cilium-associated genes in distal airway stem cell populations in IPF lung.

Major Task 1: Markers of ciliogenesis (ARL13B and FOXJ1), MUC5B and MMP7 will be co-localized with basal cell markers (KRT5, KRT14, and p63) and Wnt signaling marker β-catenin following injury (scheduled for months 0-36; 50% complete).

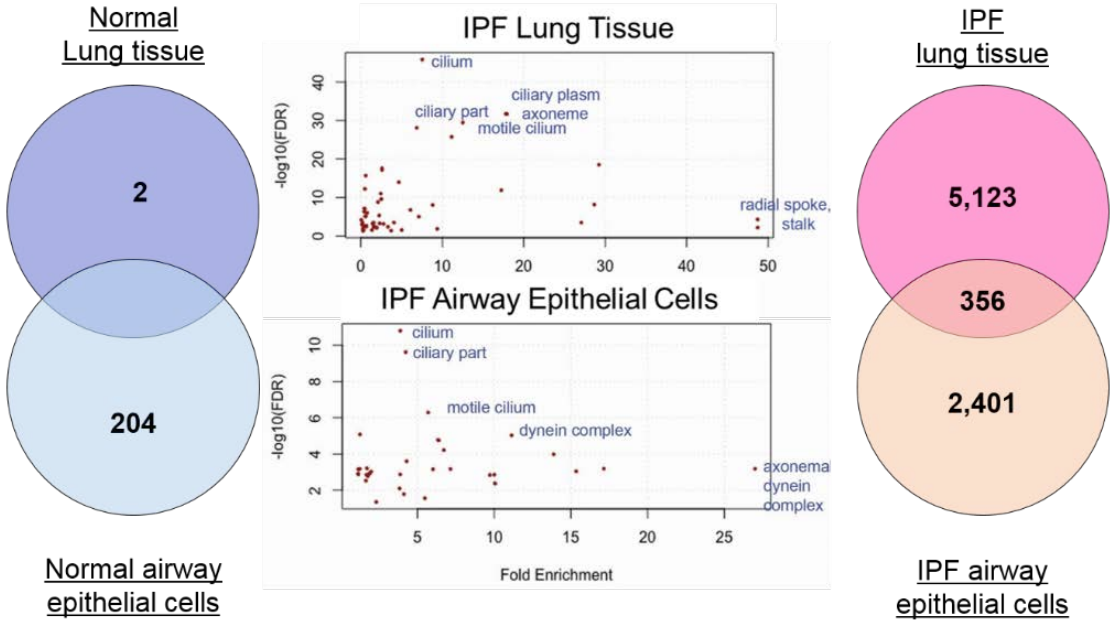


Using the same panel of markers for ciliogenesis as in Aim 1, we continue to quantify the extent of ciliogenesis in airway epithelia of IPF and control subjects. There are no differences in MYB, MYB/FOXJ1 or FOXJ1 positive cells in large airways by disease or genotype. Analysis of small airways revealed significantly increased numbers of FOXJ1 positive cells in GT/TT compared to GG IPF subjects ($p<0.0001$). We do not have enough data analyzed yet in controls to compare IPFs to controls and are in the process of completing these counts. We are in the process of counting multiciliogenesis marker positive cells in the airway epithelium lining honeycomb cysts in IPF subjects.

Major Task 2: Measure expression of cilium genes identified in Aims 1-2 in DASCs from IPF and control lungs with and without Muc5b promoter variant (scheduled for months 0-42; 25% complete).

We have continued to isolate and cryopreserve airway cells from IPF explanted lungs and non-diseased donor lungs. We currently have 10 IPF and 10 non-diseased samples banked.

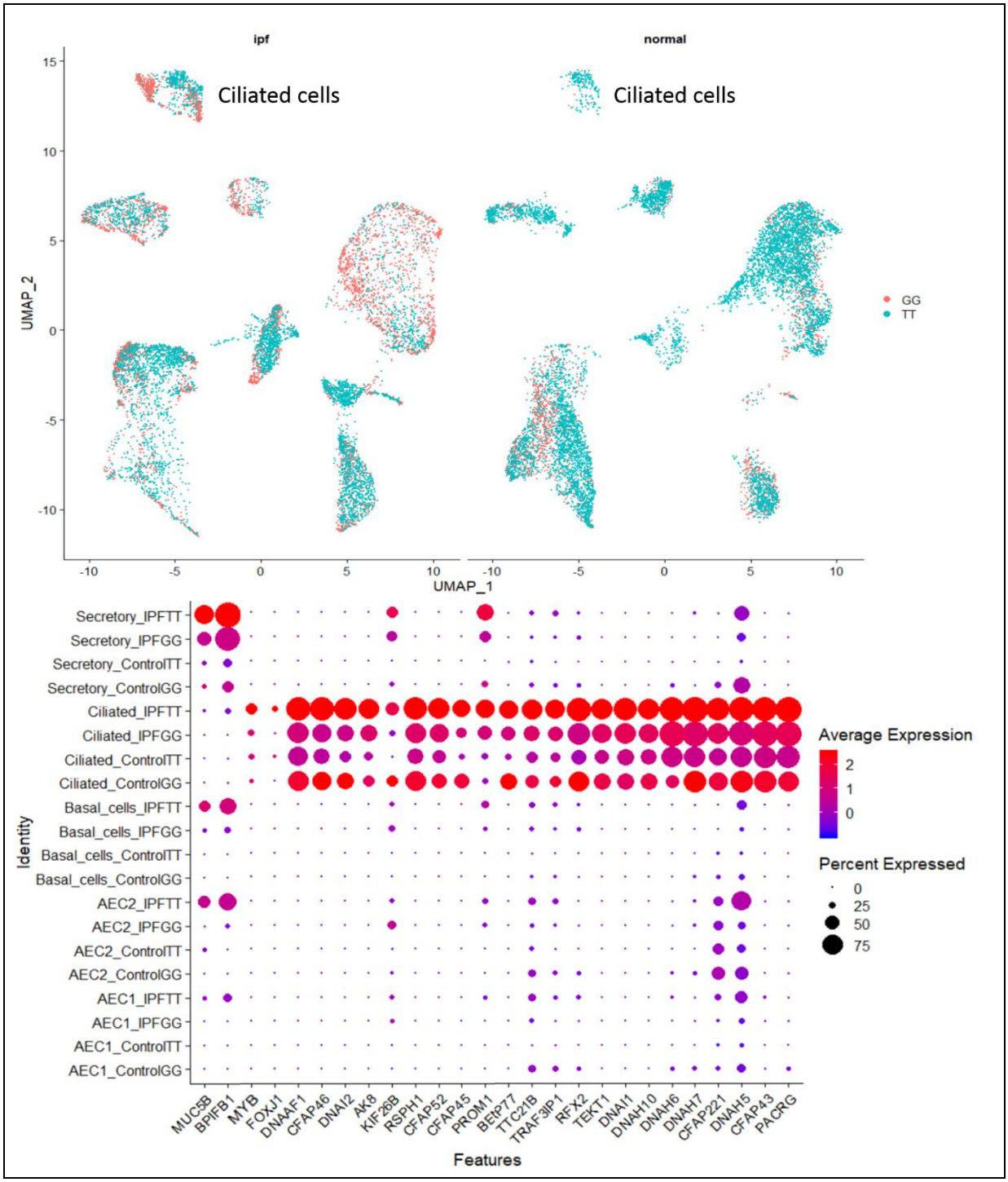
We have performed bulk RNA-seq on fresh cells from 10 IPF and 10 control lungs. We analyzed the transcriptome data, along with our data from whole lung tissue, to identify genes that are highly correlated with MUC5B expression. In normal airway epithelial cells, 203 genes were associated with *MUC5B* gene expression (FDR<0.05) after adjustment for age, sex, and smoking status. Using the same model and criteria, only two genes were associated with *MUC5B* gene expression in normal



lung tissue (*SLC25A17, BPIFB1*: FDR<0.05). In contrast 2,401 genes were associated with *MUC5B* gene expression in IPF airway cells and 5,123 in IPF lung tissue (FDR < 0.05) after adjustment for age. sex and smoking status. 500 genes with the strongest correlations to *MUC5B* in IPF airway cells were examined for enrichment in gene ontology (GO) cellular component categories; four of the top five cellular component pathways enriched were cilia related (cilium [FDR $1.57x10^{-11}$], ciliary part [FDR $2.38x10^{-10}$], motile cilia [FDR $5.12x10^{-7}$], and dynein complex [FDR $9.2x10^{-6}$Similarly, the 500 genes with strongest correlations to *MUC5B* in IPF lung tissue were analyzed for GO cellular component enrichment; the top five cellular component pathways enriched were related to cilia (cilium [FDR $1.47x10^{-46}$], ciliary plasm [FDR $1.79x10^{-32}$], axoneme [FDR $2.00x10^{-32}$], motile cilia [FDR $3.36x10^{-30}$], and ciliary part [FDR $7.76x10^{-29}$]. In IPF, lung tissue and airway cells had 365 overlapping genes significantly associated with *MUC5B* expression and the top two enriched pathways for these overlapping genes were also cilia related (cilium, FDR $1.14x10^{-19}$; ciliary part, FDR $3.56x10^{-18}$).

We have also cultured and differentiated on air-liquid interface cells from 3 IPF and 3 control lungs, and performed bulk RNA-seq on cells that have bene on ALI for 4, 8, 12, and 14 days to characterize the timecourse of expression of ciliogenesis genes. This analysis reveals decreased ciliogeenesis in IPF at early timepoints (days 4 and 8) when cells are not differentiated, similar ciliogenesis between cases and controls at day 12, and increased ciliogenesis in IPF in fully differentiated cells at day 14. These results are in agreement with the results in animal models in Aim 1. We will next perform sequencing of cells from additional IPF and control subjects to enable analysis by MUC5B genotype.

We have also isolated single nuclei from frozen tissue of four subjects (IPF GG, IPF TT, control GG, and control TT). Single nuclear RNA sequencing (snRNA-seq) revealed dramatic changes in multiple cell populations, including the ciliated cell cluster based on disease and genotype. Differential expression analysis across these cell clusters revealed an increase in expression of MUC5B and its regulator BPIFB1 in IPF TT individuals secretory, basal, and alveolar type 2 cells. In ciliated cells, IPF TT has increased expression of MYB and FOXJ1 as well as a number of genes involved in ciliogenesis.



Biostatistics Core

As from the beginning of the project, Dr. Fingerlin is available to all project investigators as it relates to study design or other questions. In response to lower enrollment than anticipated in Project 1, Dr. Fingerlin worked with Dr. Schwartz to evaluate the impact of the changes on power for the primary outcomes of the study, which were minimal. This information was included in discussions that Dr. Schwartz had with colleagues and officials at the DoD. The summary of the conclusions of those power analyses are included here:

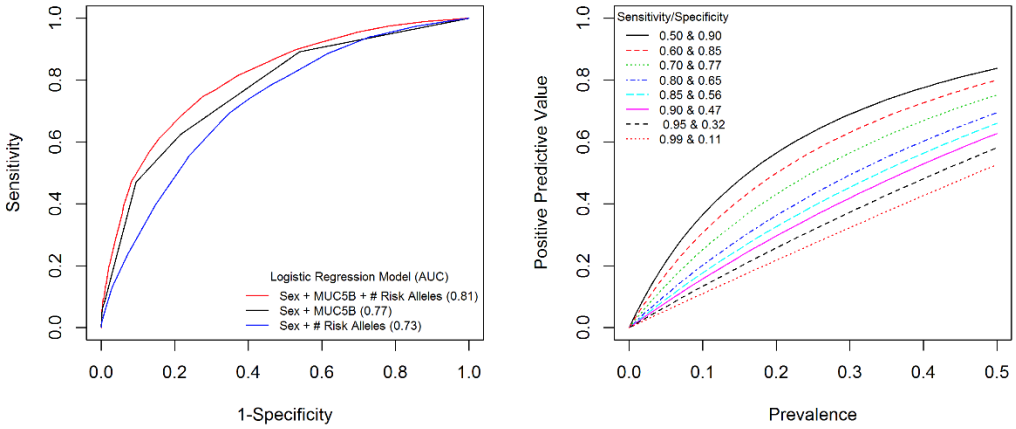
Summary of revised power: The original calculations assumed 500 FDRs of each type (500 Familial and 500 Sporadic), with the familial FDRs already recruited. The follow-up for Familial IPF FDRs is consistent with estimates from application. The changes in the sporadic FDR recruitment change the over-all ability to bound the sensitivity and specificity very little given that 1) they are not used for derivation in Aim 2, 2)

the rate of PrePF is three times what we expected (thereby reducing the imbalance between PrePF and no PrePF), and 3) the sporadic FDR progressors were combined with the familial FDRs for Aim 3.

The core, in collaboration with other projects, has completed a number of genetic analyses that have refined our understanding of the location of both common and rare variants that contribute to risk of pulmonary fibrosis, and that are used in the derivation and validation of the predictive models for Aims 2 and 3 of Project 1 that inform all of the projects. We present below the findings that have been reported in Moore et al 2019:

Common Variants

The strongest common IPF risk variant is still the previously-identified MUC5B promoter variant, rs35705950, with an OR of 5.45 (95% CI: 4.91-6.06) for one copy of the risk allele and 18.68 (95% CI: 13.34-26.17) for two copies of the risk allele (p=9.60 x 10-295). Testing only significant common variants within each GWAS resequencing region, there were no additional common variants that were statistically significantly associated with IPF after adjusting for the top variant in that region. In addition, these variants are associated with similar risk in familial and sporadic IPF. The potential classification and prediction utility of these variants are explored via the receiver operating characteristic curves and representative positive predictive values for using sex, the MUC5B promoter variant (rs35705950), and the number of other risk variants. In comparison to other common variants, rs35705950 has the best potential for risk prediction (Figure 1). Since several of these variants have been identified previously in independent populations, the ROC curves and positive predictive values are not as susceptible to estimation bias that is usually present when estimating such values from the same sample as that used for association testing. In particular, only 226 cases overlap between our original GWAS and this study.



Grouped Rare Variant Analyses

In total, we identified 12 rare-variant window-based sets with evidence of independent association with IPF (Table). Ten window-based sets in regions with a common signal were significant after adjusting for the top common variant in the region (Table). The most significant window in the *TERT* gene contained 27 variants with high or moderate functional impact and the most significant window in the *RTEL1* gene contained 20 high or moderate impact variants.

Table

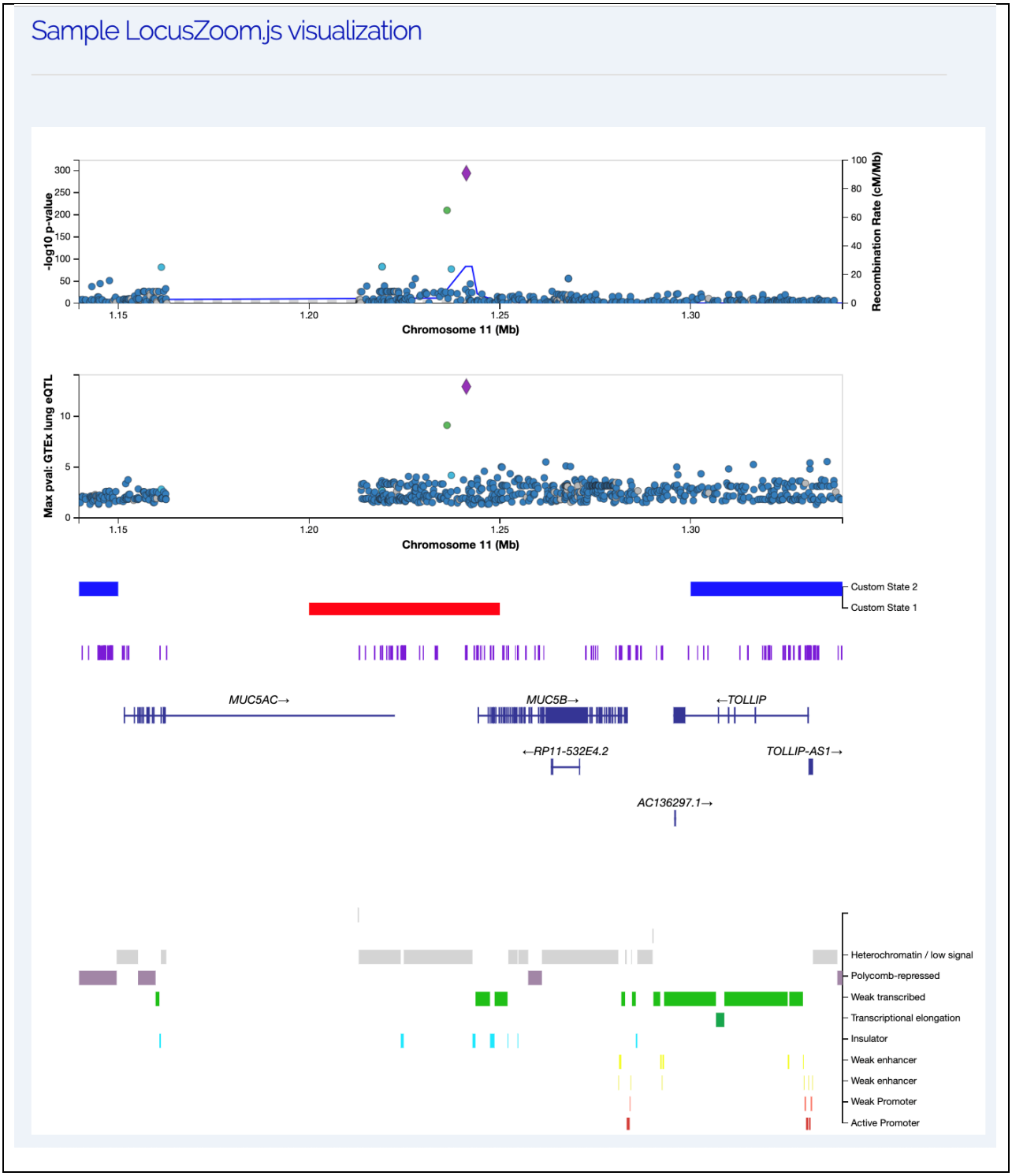
Nearest Gene[a]	Annotation	# Putative Functional variants[b]	# Windows in region	p-value[c]	p-value[d]
FAM13A	Intronic	0	615	-	0.0360
FAM13A	Intronic, exonic	1	615	8.4×10^{-6}	8.31×10^{-6}*
FAM13A	Intronic, exonic	1	615	8.9×10^{-5}	0.0001*

TERT	Upstream, 5'UTR, exonic, intronic	27	345	1.7×10^{-14}	9.21×10^{-16}*
TERT	Upstream, 5'UTR, exonic, intronic	8	345	-	0.0083
MIR4457	Downstream	0	345	0.001	0.0011*
MIR4457	Downstream	0	345	0.001	0.0011*
CLPTM1L	Exonic, 3'UTR, Intronic	1	345	0.007	0.0236
RP13-870H17.3	Downstream	0	1294	8.8×10^{-4}	0.0386
MCF2L	Intronic	0	885	0.02	0.0312
RNPS1	Intronic	0	165	0.02	0.0162
RTEL1	Exonic, intronic	20	151	0.02	0.0215

eQTLs in Significant Regions of Association

In addition to rs35705950 on chr11, three of the significant common variants in other regions are expression quantitative trait loci (eQTLs) for expression of genes in lung tissue from the GTEx consortium. On chr6, rs2076295 is an eQTL for *DSP*, as we have previously reported. On chr15, rs35700143 is an eQTL for *BAHD1*, a nuclear protein that promotes heterochromatic gene silencing (20) and that when repressed, contributes to the induction of interferon (IFN)-stimulated genes. And on chr19, rs12601495 is an eQTL for *DPP9*.

In addition to the statistical analyses, we have developed several tools to be used internally for visualization of results. We have completed a prototype of a visualization tool for co-displaying our genetic and molecular work with other information available in public databases like ENCODE. We include a screen shot below of the tool, which will allow easy display of the results from each of the projects as appropriate. In the example given, we display our resequencing-based association results in the top panel, GTEx expression quantitative trait loci associations in lung tissue, mock tracks showing targeted mutagenesis regions used for identifying transcription factor binding sites, and several ENCODE tracks.



c. What opportunities for training and professional development has the project provided?

- *If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."*
- *Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.*

Project 1: Nothing to report
Project 2: Nothing to report

Project 3: Nothing to report
Project 4: Dr. Kim completed a hands-on workshop Applications of Organoid Technology organized by offered by MDI Biological Laboratory in partnership with Hubrecht Organoid Technology (HUB) in May 2019.
Biostatistics Core: Nothing to report

d. How were the results disseminated to communities of interest?

- *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
- *Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.*

Project 1: CT scan results are shared with participants following central review of their imaging at the University of Colorado.
Project 2: results shared via local seminars to faculty, fellows and students
Project 3: Findings were presented at the 2019 American Thoracic Society Conference, a 2019 Gordon Research Conference, and in five publications.

- ***Ann Am Thorac Soc.*** Mucociliary Defense: Emerging Cellular, Molecular, and Animal Models.2018. PMID: 30431350
- ***Nature Communications***, Muc5b overexpression causes mucociliary dysfunction and enhances lung fibrosis in mice. 2018. PMID: 30560893
- ***Am J Respir Crit Care Med***. Dawn of a New Era in the Diagnosis and Treatment of Airway Mucus Dysfunction. 2019. PMID: 30252497
- ***JCI Insight***, Syndecan-1 promotes lung fibrosis by regulating epithelial reprogramming through extracellular vesicles. 2019. PMID: 31393853
- ***Am J Respir Cell Mol Biol***. Muc5b Enhances Murine Honeycomb-like Cyst Formation. 2019. PMID: 31573335.

Project 4:
Dr. Kim presented a poster at the Gordon Cilia, Mucus and Mucociliary Interactions Research Conference held Feb 17-22, 2019.
Biostatistics Core: Nothing to report

e. What do you plan to do during the next reporting period to accomplish the goals?

- *If this is the final report, state "Nothing to Report."*
- *Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.*

Project 1:

- Referral, recruitment, and enrollment of research participants will continue
- Continue to administer informed consent to interested people and enroll participants in the study. For enrolled participants, continue to complete study procedures (blood draw, CT scan)
- Phenotyping of participants and sharing CT scan results will continue in next reporting period

Project 2:
During the next reporting period, we will use bioinformatics and functional assays to further define candidate factors that regulate MUC5B. We will use the data from the PRO-SEQ experiments as a basis for chromatin conformation capture assays, a key piece of Aim 2. We will publish a paper on our analysis of the chromatin structure of the MUC5B -3kB enhancer. We will expand on our findings that GCF represses MUC5B through DNA binding assays and biochemical approaches.
Project 3:
We will continue on Aim 1, Major Tasks 2 and 3. To accomplish this we are performing mucociliary transport studies in all mouse lines and in additional human cells. We are also preparing an additional manuscript on transport function and mucus gel structure.

We are also continuing Aim 2 using mouse and human samples to determine mucin glycosylation and how this affects mucus gel structure, and mucous cell biosynthetic component levels.

For Aim 3, we are breeding mice onto appropriate backgrounds and assigning them to challenge groups. We have begun challenging them with bleomycin and will conduct PF studies in them.
Project 4:
Aim 1: Determine the effect of Muc5b concentration on expression of cilium-associated genes in distal airway stem cell populations following injury in mice.
Major Task 3: Identify changes in cilium gene expression in isolated DASC populations at multiple timepoints following injury (scheduled for months 3-18; 50% complete).

We will finish analysis of single cell RNA-seq data in Muc5b Tg mice treated with bleomycin. We will iisolate Krt5+ cells after injury with bleomycin and H1N1 and perform gene expression analysis.
Major Task 4: Publication of findings from Aim 1 (scheduled for months 18-24; 75% complete)
We plan on submitting the publication that describes the results from Aim 1 by the end of 2019.

Aim 2: Demonstrate that changes in cilium gene expression in airway progenitor cells affect injury/repair and fibrosis.
Major Task 1: Establish NHBE cell cultures, optimize lenti-shRNA and lenti-ORF protocols, and treatment concentrations (scheduled for months 0-18; 50% complete).
We will finish optimization of lenti-shRNA and lenti-ORF protocols by performing knockdown and overexpression of control genes.

Major Task 2: Inhibit and overexpress cilium genes, measure injury/repair, regeneration, and Wnt signaling (scheduled for months 18-36; 5% complete).

Major Task 3: Determine the influence of cilium gene deletion on injury/repair, lung regeneration, and fibrosis in mice (scheduled for months 3-39; 25% complete).
We are currently breeding mice to conditionally delete Ift88 in Scgb1a1-derived cells to determine contribution of these progenitor cells to enhanced muclticiliogenesis phenotype we have observed. We have requested additional breeders for Arl13b line from Dr. Caspary and are also importing Gemc1-/- mice for this Aim.

Aim 3: Determine the contribution of the MUC5B promoter variant on expression of cilium-associated genes in distal airway stem cell populations in IPF lung.
Major Task 1: Markers of ciliogenesis (ARL13B and FOXJ1), MUC5B and MMP7 will be co-localized with basal cell markers (KRT5, KRT14, and p63) and Wnt signaling marker β-catenin following injury (scheduled for months 0-36; 50% complete).
We will quantify markers of multiciliogenesis in oneycomb cyst regions.
Major Task 2: Measure expression of cilium genes identified in Aims 1-2 in DASCs from IPF and control lungs with and without Muc5b promoter variant (scheduled for months 0-42; 25% complete).
We will continue to collect cells from IPF and control lung tissue and perform single cell RNA-seq on additional subjects.

Biostatistics Core: nothing to report

2. Impact

Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:

a. What was the impact on the development of the principal discipline(s) of the project?

- *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
- *Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).*

Project 1:
This study's enrollment of healthy relatives of people with sporadic IPF is spreading the idea that IPF can run in families even when there is no known family history, which is important for explaining risk of IPF. As we continue to reach out to physicians about patient recruitment, we share the knowledge that genetics are a key risk factor for IPF.
Project 2:
Our finding that analyzing new transcription in high resolution allows specific genetic mutations to be associated with regulating a given gene has implications for other genetic studies of human disease.
Project 3:
We have championed a concept that is driving the pulmonary fibrosis field in a new direction. Along with other Program Project Grant teams, we are demonstrating that mucociliary dysfunction is an important and treatable phenomenon in lung fibrosis.
Project 4:
Our work over the past year has identified critical timepoints at which ciliogenesis is overactive following lung injury, in the context of overproduction of the airway mucin MUC5B. We have also demonstrated

changes in cilium gene expression in isolated airway epitehlia and at the single cell level in lung tissue from IPF and control subjects.

Biostatistics Core: nothing to report

b. What was the impact on other disciplines?

- *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
- *Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.*

Project 1: Nothing to report
Project 2: Nothing to report
Project 3: Our findings are carrying a concept of mucous cell proteostasis that is being tested in acute lung injury and asthma.
Project 4: Nothing to report
Biostatistics Core: Nothing to report

c. What was the impact on technology transfer?

- *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
- *Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:*
    - *transfer of results to entities in government or industry;*
    - *instances where the research has led to the initiation of a start-up company; or*
    - *adoption of new practices.*

Project 1 – nothing to report
Project 2 – nothing to report
Project 3 – nothing to report
Project 4 – nothing to report
Biostatistics Core – nothing to report

d. What was the impact on society beyond science and technology?

- *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
- *Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:*
    - *improving public knowledge, attitudes, skills, and abilities;*
    - *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
    - *improving social, economic, civic, or environmental conditions.*

Project 1 – nothing to report
Project 2 – nothing to report
Project 3 – nothing to report
Project 4 – nothing to report
Biostatistics Core – nothing to report

3. Changes/Problems

If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:

a. Changes in approach and reasons for change

- *Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.*

Project 1: No significant changes are anticipated. We will continue to emphasize physician and patient outreach to improve recruitment into the study.
Project 2: Based on our finding that the MUC5B -3kB region is HyperChIPable, we continue to employ a variety of complimentary approaches to define molecular control of this region. This refinement to our technical approach does not significantly change the scope of the proposal or objectives.
Project 3: We generated a conditional St3gal3 knockout line that will be used in studies for Aim 3.

We generated a C57BL/6 line using CRISPR/Cas9 targeting. The antiserum used to test Ern2 protein levels was discontinued. We obtained an alternate, but it did not work well. We are independently re-making the "good" one that was discontinued.
Project 4:
We will use Gemc1-deficient mice (EMBO Rep. 2016;17:400-13) to interrupt multiciliogenesis at a later timepoint than Ift88 and to provide alterantive to Arl13b mice should we continue to have issue with their breeding.

Biostatistics Core: nothing to report

b. Actual or anticipated problems or delays and actions or plans to resolve them

- *Describe problems or delays encountered during the reporting period and actions or plans to resolve them.*

Project 1: Recruitment has picked up in the last few months of the reporting period. We received IRB approval from more recruitment sites in the past year. We are also reaching out more consistently and more frequently to referral sites to increase the pace of referrals. We are nearly finished with a study recruitment website to allow potential participants to self-refer to the study
Project 2: The realization that the MUC5B -3kB region is open and hyperChIPable has been informative but has also delayed progress on our basic understanding of the SNP.
Project 3: See above 3.a
Project 4: While we are slightly delayed in completing gene expression studies on cells isolated from mouse lungs in Aim 1, we are a little bit ahead of the schedule in single cell analysis of human lung tissue in Aim 3.
Biostatistics Core: nothing to report

c. Changes that had a significant impact on expenditures

- *Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.*

Project 1: no changes
Project 2: no changes
Project 3: We conducted single-cell RNA-sequencing to identify novel membrane markers for lung epithelia. This cost was shared among investigators in the program project grant and on other grants to limit the impact of the costs on Project 3.
Project 4: no changes
Biostatistics Core: no changes

d. Significant changes in the use or care of human subjects, vertebrate animals, biohazards, and/or select agents

- *Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.*

Project 1: no changes
Project 2: no changes
Project 3: New mouse lines for *St3gal3* and *Ern2*
Project 4: no changes
Biostatistics Core: no changes

e. Significant changes in use or care of human subjects

Project 1: no changes
Project 2: no changes
Project 3: no changes
Project 4: no changes
Biostatistics Core: no changes

f. Significant changes in use or care of vertebrate animals

Project 1: no changes
Project 2: no changes

Project 3: no changes
Project 4: no changes
Biostatistics Core: no changes

g. Significant changes in use of biohazards and/or select agents

Project 1: no changes
Project 2: no changes
Project 3: no changes
Project 4: no changes
Biostatistics Core: no changes

4. Products

List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

a. Publications, conference papers, and presentations

Report only the major publication(s) resulting from the work under this award.

- ***Journal publications****. List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*
- ***Books or other non-periodical, one-time publications.*** *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*
- ***Other publications, conference papers, and presentations.*** *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Project 1: Nothing to report
Project 2: Manuscript in progress
Project 3:

- ***Ann Am Thorac Soc.*** Mucociliary Defense: Emerging Cellular, Molecular, and Animal Models.2018. PMID: 30431350
- ***Nature Communications***, Muc5b overexpression causes mucociliary dysfunction and enhances lung fibrosis in mice. 2018. PMID: 30560893
- ***Am J Respir Crit Care Med***. Dawn of a New Era in the Diagnosis and Treatment of Airway Mucus Dysfunction. 2019. PMID: 30252497
- ***JCI Insight***, Syndecan-1 promotes lung fibrosis by regulating epithelial reprogramming through extracellular vesicles. 2019. PMID: 31393853
- ***Am J Respir Cell Mol Biol***. Muc5b Enhances Murine Honeycomb-like Cyst Formation. 2019. PMID: 31573335.

Project 4: Dr. Kim presented a poster at the Gordon Cilia, Mucus and Mucociliary Interactions Research Conference held Feb 17-22, 2019.

b. Website(s) or other Internet site(s)

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Project 1 – nothing to report
Project 2 – nothing to report
Project 3 – nothing to report
Project 4 – nothing to report
Biostatistics Core – nothing to report

c. Technologies or techniques

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Project 1 – nothing to report
Project 2 – nothing to report
Project 3 – nothing to report
Project 4 – nothing to report
Biostatistics Core – nothing to report

d. Inventions, patent applications, and/or licenses

Identify inventions, patent applications with date, and /or licenses that have resulted from the research. State whether an application is provisional or non-provisional and indicate the application number. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Project 1 – nothing to report
Project 2 – nothing to report
Project 3 – nothing to report
Project 4 – nothing to report
Biostatistics Core – nothing to report

e. Other products

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *biospecimen collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Project 1 – nothing to report
Project 2 – nothing to report
Project 3 – nothing to report
Project 4 – nothing to report
Biostatistics Core – nothing to report

5. Personnel Effort

Provide the following information for: (1) Site PI; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort).

Project 1

Name:	**David Schwartz, MD**
Project Role:	PI/Project Lead
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	2
Contribution to project:	Responsible for the design and execution of the study, and the day-to-day functioning, trouble-shooting, integration, training, and long-term planning of the study.
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Joyce Lee, MD**
Project Role:	Co-Investigator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	2
Contribution to project:	Longitudinally assess genetically at-risk cohorts for the appearance of autoantibodies and for the subsequent progression to clinical disease.
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Jill Norris, PhD**
Project Role:	Co-Investigator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	
Contribution to project:	1
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Marvin Schwarz, MD**
Project Role:	Co-Investigator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	1
Contribution to project:	Provide oversight for the clinical phenotyping of the subjects in this project.
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Tasha Fingerlin**
Project Role:	Co-Investigator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	1
Contribution to project:	Oversees all of the statistical analyses related to the biomarker discovery and validation work in relationship to Project 1.
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Corinne Hennessy**
Project Role:	Professional Research Assistant
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	6
Contribution to project:	Responsible for organizing, tracking, and curating the DNA and biological samples for this project, and the follow up genotyping efforts and biomarker assays (mRNA and protein).
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Rachel Bochantin**
Project Role:	Study Coordinator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	12

Contribution to project:	Serves as Study Coordinator for this project. Ms. Bochantin is responsible for coordinating the efforts of the co-investigators, acquiring all of the clinical data and making arrangements to obtain high-resolution CT (HRCT) scans, peripheral blood (DNA, RNA from PBMCs, and plasma), and pulmonary function tests (PFTs) on asymptomatic siblings of established IPF patients in Years 1-2 and the follow-up HRCT scans and PFTs in Years 3-4 on subjects with PrePF.
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Julie Powers**
Project Role:	Clinical Coordinator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	4
Contribution to project:	Overseeing clinical coordination team, completing IRB and regulatory submissions, managing referral sites and their IRB and regulatory submissions, administering informed consent with new participants, and coordinating the phenotyping process of participant CT scans and health records
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Mark Steele, MD**
Project Role:	Co-Investigator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	2
Contribution to project:	Contribute to recruitment and accrual of patients and their families at University of Colorado and phenotype enrolled participants.
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Kevin Brown**
Project Role:	Co-Investigator, MD
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	1
Contribution to project:	Coordinate the accrual of patients and their families at National Jewish Health
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Daniel Kass, MD**
Project Role:	Co-Investigator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	1
Contribution to project:	Coordinate the accrual of patients and their families at University of Pittsburgh
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Paul Wolters, MD**
Project Role:	Co-Investigator

Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	1
Contribution to project:	Coordinate the accrual of patients and their families at UCSF
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**James Loyd, MD**
Project Role:	Co-Investigator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	1
Contribution to project:	Coordinate the accrual of patients and their families at Vanderbilt University
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Steven Rowe**
Project Role:	Co-Investigator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	1
Contribution to project:	Direct mucociliary clearance enrollment, study conduct, and analysis
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Victor Thannickal**
Project Role:	Co-Investigator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	1
Contribution to project:	Assists with patient identification and pre-screening. Will be involved in analysis when data are complete.
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Ginger Reeves**
Project Role:	Clinical Trials Administrator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	1
Contribution to project:	Recruits subjects and helps perform MCC imaging studies
Funding support:	*Complete only if the funding support is provided from other than this award*

Project 2

Name:	**Anthony Gerber**
Project Role:	Co-Project Lead
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	2
Contribution to project:	Directing research team
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Sarah Sasse**

Project Role:	Co-investigator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	2
Contribution to project:	Performed and analyzed experiments
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Fabienne Gally**
Project Role:	Co-investigator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	5
Contribution to project:	Performed and analyzed experiments
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Sean Colgan**
Project Role:	Co-Project Lead
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	2
Contribution to project:	Directing research team
Funding support:	*Complete only if the funding support is provided from other than this award*

Project 3

Name:	**Christopher Evans**
Project Role:	Project Lead
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	2
Contribution to project:	oversight of experimental design, performance, and analysis in Project 3
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Naoko Hara**
Project Role:	Professional Research Assistant
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	6
Contribution to project:	Animal husbandry and analyses of mucins
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Michael Cross**
Project Role:	Professional Research Assistant
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	5
Contribution to project:	Animal husbandry and challenges
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Breanna Symmes**
Project Role:	Postdoctoral fellow

Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	6
Contribution to project:	Investigating differential glycosylation of airway mucins
Funding support:	*Complete only if the funding support is provided from other than this award*

Project 4

Name:	**Ivana Yang, PhD**
Project Role:	Project Lead
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	2
Contribution to project:	Design and execution of the study, the day-to-day functioning, trouble-shooting, integration, training, and long-term planning of the study; oversight for Dr. Eunjoo Kim and Ms. Elizabeth Davidson; actively participates in data analysis, data interpretation, and manuscript preparation; conducts weekly meetings with the project personnel
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Oliver Eickelberg, MD**
Project Role:	Co-Investigator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	1
Contribution to project:	Provide expertise in procedures and methods for isolation and study of specific cell populations from IPF lung explants and biopsies; supervision to Dr. Yan Hui on isolation of cell populations
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Melanie Königshoff, MD**
Project Role:	Co-Investigator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	1
Contribution to project:	Provide expertise in Wnt signaling and lung regeneration following injury; supervision to Dr. Yan Hui on Wnt investigation in the proposal
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Eunjoo Kim, PhD**
Project Role:	Postdoctoral Fellow
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	12
Contribution to project:	Breeding of Ift88-Krt5 and Arl13b-Krt5 CKO animals; i.t bleomycin, and i.n. H1N1 treatments; immunofluorescence analysis of animal tissue; hydroxyproline assays
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Yang Hui, PhD**

Project Role:	Postdoctoral Fellow
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	6
Contribution to project:	Isolation of distal airway stem cell populations from human and mouse tissue, Wnt signaling assays
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Elizabeth Davidson**
Project Role:	Professional Research Assistant
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	3
Contribution to project:	NHBE cell culture; immunofluorescence analysis on human and animal tissue
Funding support:	*Complete only if the funding support is provided from other than this award*

Biostatistics Core

Name:	**Tasha Fingerlin, PhD**
Project Role:	Biostatistics Core Director
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	1
Contribution to project:	Oversees all of the statistical analyses related to the biomarker discovery and validation work in relationship to Project 1.
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Kelsey Anderson**
Project Role:	Computer Programmer
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	3
Contribution to project:	Responsible for designing and implementing database structures that allow the individual projects to efficiently deposit and retrieve study data, as well as coordinating the integration of systems in such a way as to preserve individual study features while allowing efficient integration of data across projects.
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Sean Jacobson**
Project Role:	Junior Biostatistician
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	7
Contribution to project:	Responsible for day-to-day analytic activities for all projects, with duties determined by Dr. Fingerlin in response to investigator needs and priorities. Works directly with Dr. Fingerlin to implement summary reporting, project analyses and data reports for Project Directors and works with the computer programmer to develop the data sets and implement data cleaning and reporting algorithms.
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Camille Moore**
Project Role:	Senior Biostatician
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	1
Contribution to project:	Bio-analysis lead work for the biostatistic core
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Brian Vestal**
Project Role:	Senior Statistician
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	4
Contribution to project:	Bio-analysis lead work for the biostatistic core
Funding support:	*Complete only if the funding support is provided from other than this award*

Administrative Core

Name:	**David Schwartz, MD**
Project Role:	Administrative Core Lead
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	1
Contribution to project:	Responsible for the scientific coordination, direction of research emphasis, and administrative activities of the Program
Funding support:	*Complete only if the funding support is provided from other than this award*

Name:	**Soumontha Chanthaphonh**
Project Role:	Administrator
Research Identifier (e.g. ORCID ID)	
Nearest person month worked:	2
Contribution to project:	Manage the fiscal and administrative aspects of the Program and coordinate matters with participating departments, the University of Colorado, the subcontract sites, and the Department of Defense
Funding support:	*Complete only if the funding support is provided from other than this award*

a. Has there been a change in the active other support of the Site PI or senior/key personnel since the last reporting period?

- *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
- *If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.*

Project 1:
The following for Drs. David Schwartz and Tasha Fingerlin has been completed:
R01HL097163
NIH/NHLBI
Role of Genetics in Idiopathic Pulmonary Fibrosis (Schwartz, Fingerlin)

08/01/2011-06/30/2019
2.4 calendar

The following grant for Dr. David Schwartz has been completed:
R33HL120770
NIH/NHLBI
Functional Genetics in Idiopathic Pulmonary Fibrosis (Schwartz)
08/08/2014-06/30/2019
1.2 calendar

Dr. Tasha Fingerlin was awarded Multi-PI project R01HL142049, Novel Integrative Approaches for Disease Phenotyping, Utilizing Radiomics in Sarcoidosis, with 1.8 calendar months of effort. The addition of this project will have no effect on her performance on this research project.

Dr. Joyce Lee has the following new active clinical trials:
Study of Pulmonary Rehabilitation In Nintedanib Treated Patients with IPF: Improvements in Activity, Exercise Endurance Time, and QoL
Boehringer Ingelheim Pharmaceuticals, Inc.
12/01/2018-11/30/2021

Novel Biomarkers in Rheumatoid Arthritis Associated Interstitial Lung Disease
Boehringer Ingelheim Pharmaceuticals, Inc.
08/28/2019-08/27/2024

A Phase 3, randomized, double-blind, parallel-group, placebocontrolled multicenter study to evaluate the efficacy and safety of two doses of GLPG1690 in addition to local standard of care for minimum 52 weeks in subjects with idiopathic pulmonary fibrosis.
Galapagos NV
08/21/2019-08/20/2024

Project 2:
Dr. Fabienne Gally was awarded an NIH R01. Drs. Gally, Gerber, and Sasse each have the follow level of effort committed to the new project. This new project does not interfere with the current DoD project.

R01HL141264 (Gally)
Role of FABP5 in COPD Exacerbations
06/15/2019 – 04/30/2023
3.0 calendar

Gerber, Anthony (Co-Investigator)
R01HL141264 (Gally)
06/15-2019 – 04/30/2023
0.6 calendar

Sasse, Sarah (Faculty)
R01HL141264 (Gally)
06/15/2019 – 04/30/2023
1.2 calendar

Project 3
New active grants for Dr. Christopher Evans:
EVANS1810 (Evans)
Cystic Fibrosis Foundation
Requirements for Polymeric Mucin Disulfide Assembly in Vivo
11/01/2018 – 10/31/2020
1 calendar
R01HL080396 (Evans) RENEWAL
NIH/NHLBI
Role of Mucin in Lung Homeostasis and Pathophysiology
04/01/2019-03/31/2024
4 calendar

Dr. J De Andrede left UAB for Vanderbilt University. Dr. V. Thannickal replaced his role on the project after being fully trained on study procedures and conduct. He is an expert in IPF and fully capable of completing study duties.

Administrative Core
Ms. Soumontha Chanthaphonh replaced Ms. Sarah Handly as Administrator in November 2018

b. What other organizations were involved as partners?

- *If there is nothing significant to report during this reporting period, state "Nothing to Report."*
- *Describe partner organizations - academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) - that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed. Provide the following information for each partnership:*
  - *Organization Name:*
  - *Location of Organization: (if foreign location list country)*
  - *Partner's contribution to the project (identify one or more)*
    - *Financial support;*
    - *In-kind support (e.g., partner makes software, computers, equipment, etc., available to*
    - *project staff);*
    - *Facilities (e.g., project staff use the partner's facilities for project activities);*
    - *Collaboration (e.g., partner's staff work with project staff on the project);*
    - *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
    - *Other.*

Nothing to report

6. Appendices

Attach all appendices that contain information that supplements, clarifies or supports the text.
Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.