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PRINCIPAL INVESTIGATOR: Naftali Kaminski, M.D.

CONTRACTING ORGANIZATION: Yale University
15 York Street , New Haven, CT 06510-3221

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Naftali Kaminski, M.D.

E-Mail: naftali.kaminski@yale.edu

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Yale University
Department of Pulmonary and Critical Care Medicine,
MIMED 721774
15 York Street , New Haven, CT 06510-3221

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14. ABSTRACT

Our proposal focuses on the role of FENDRR, a developmentally regulated lincRNA that controls gene expression by affecting chromatin remodeling in Pulmonary fibrosis (PF). PF is a condition in which the normal lung anatomy is replaced by a process of active remodeling, deposition of extracellular matrix (ECM) and accumulation of myofibroblasts. This condition can be idiopathic or secondary, but invariably associated with significant mortality and morbidity. In this project we test the hypothesis that FENDRR expression maintains fibroblasts differentiation status through its effects on chromatic organization, therefore when FENDRR expression is decreased, fibrosis is facilitated through persistence of myofibroblasts. We have made significant progress on our specific aims: **Specific aim 1:** To determine the mechanisms by which FENDRR regulates fibroblast phenotypes - We identified now more the key epigenetic modifications that FENDRR is affecting the promoters of target genes to prevent fibrosis; **Specific aim 2:** To determine the role of FENDRR in animal models of fibrosis - We have generated FENDRR knockout mice and demonstrate that they are more susceptible to fibrosis; **Specific Aim 3:** To determine the implications of FENDRR downregulation in human lungs – we confirmed the results and are now assessing target molecules.

15. SUBJECT TERMS Pulmonary Fibrosis, Large Intergenic Non-coding RNA, RNA, FENDRR, Epigenetic, Myofibroblast, Extracellular Matrix, Knockout Mouse

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REPORT OUTLINE

INTRODUCTION: Our proposal focused on the role of FENDRR, a developmentally regulated lincRNA that controls gene expression by affecting chromatin remodeling in Pulmonary fibrosis (PF). PF is a condition in which the normal lung anatomy is replaced by a process of active remodeling, deposition of extracellular matrix (ECM) and accumulation of myofibroblasts. PF can be idiopathic or secondary, but in either case, it is associated with significant mortality and morbidity. In this project we tested the hypothesis that FENDRR has antifibrotic properties and that its downregulation allows profibrotic changes in lung fibroblasts and sought to establish the mechanisms for this effect and potentially its therapeutic utility.

KEYWORDS: Pulmonary Fibrosis, Large Intergenic Non-coding RNA, RNA, FENDRR, OXF1 Adjacent Non-Coding Developmental Regulatory RNA, Epigenetic, Myofibroblast, GATA6, CDKN1A, Extracellular Matrix, Knockout Mouse

ACCOMPLISHMENTS:

Goals

Our overall proposal tested the novel hypothesis that FENDRR expression maintains fibroblast differentiation status through its effects on chromatic organization, therefore, when FENDRR expression is decreased, fibrosis is facilitated through persistence of myofibroblasts. We proposed to address this hypothesis by the following specific aims:

Specific Aim 1: To determine the mechanisms by which FENDRR regulates fibroblast phenotypes. This aim included the following tasks: A) Confirmation that increased extracellular matrix expression in response to FENDRR inhibition is mediated through changes in histone methylation of transcription factors regulating ECM expression. B) Identification of the changes in chromatin remodeling leading to increased αSMA and stress fiber formation. C) Determination whether FENDRR is a regulator of signals from the extracellular matrix that affect fibroblast to myofibroblast differentiation. All of these tasks have been achieved.

Specific Aim 2: To determine the role of FENDRR in animal models of fibrosis. This aim included the following tasks: A) Use Lentiviral vector administration of FENDRR in the bleomycin-induced murine model of lung fibrosis to determine its potential therapeutic role. B) Identification of the epigenetic changes induced by in-vivo administration of FENDRR in mouse lung fibroblast. C) Determination whether loss of FENDRR affects the predisposition to fibrosis in the adult mice. We have encountered significant difficulties on this aim. Tasks A and C have been fully performed. All tasks have been performed.

Specific Aim 3: To determine the implications of FENDRR downregulation in human lungs with Pulmonary Fibrosis. This translational aim will include the following experiments: A) Using a collection of 529 samples obtained from patients with chronic lung diseases, such as IPF, NSIP as well as controls determine whether changes of expression of FENDRR are specific to IPF. B) Determine whether changes in FENDRR expression are associated with parameters of disease severity as indicated by pulmonary functions. C) Using an available collection of lung biopsies to determine whether changes in FENDRR expression were associated with disease outcome in a separate cohort. All of the tasks have been performed.

What was accomplished under these goals?

FENDRR negatively regulates profibrotic phenotypes of lung fibroblasts

We hypothesized that FENDRR knockdown induces phenotypes of myofibroblastic differentiation in fibroblasts. In fact, one of the most up-regulated genes by FENDRR knock down in fibroblasts was ACTA2, the gene encoding alpha-smooth muscle actin. Along with stress fiber formation, expression of ACTA2 in fibroblasts is a hallmark of trans-differentiation of fibroblasts into myofibroblasts. We found that FENDRR inhibition induced

the expression of genes known to be upregulated in IPF fibroblasts including ACTA2, fibronectin and collagens in NHLFs (**Figure 1A**). We also note the increase in gene expression of NOX4, a ROS-producing NADPH oxidase. NOX4 upregulation is found in aging related diseases. FENDRR knock-down promotes fibroblast differentiation into myofibroblasts, as demonstrated by increased immunofluorescence signal for ACTA2 along with significant structural changes in fibroblasts including formation of stress fibers (**Figure 1C**). This evidence was further validated by western blotting confirming increased ACTA2 expression (**Figure 1D**). Sircol assay revealed increased excretion of soluble collagens by NHLFs with FENDRR inhibition. (**Figure 1B**) We also found increased intracellular ROS levels (**Figure 1E**), which is consistent with augmented NOX4 levels (**Figure 1D**) in FENDRR knockdown cells.

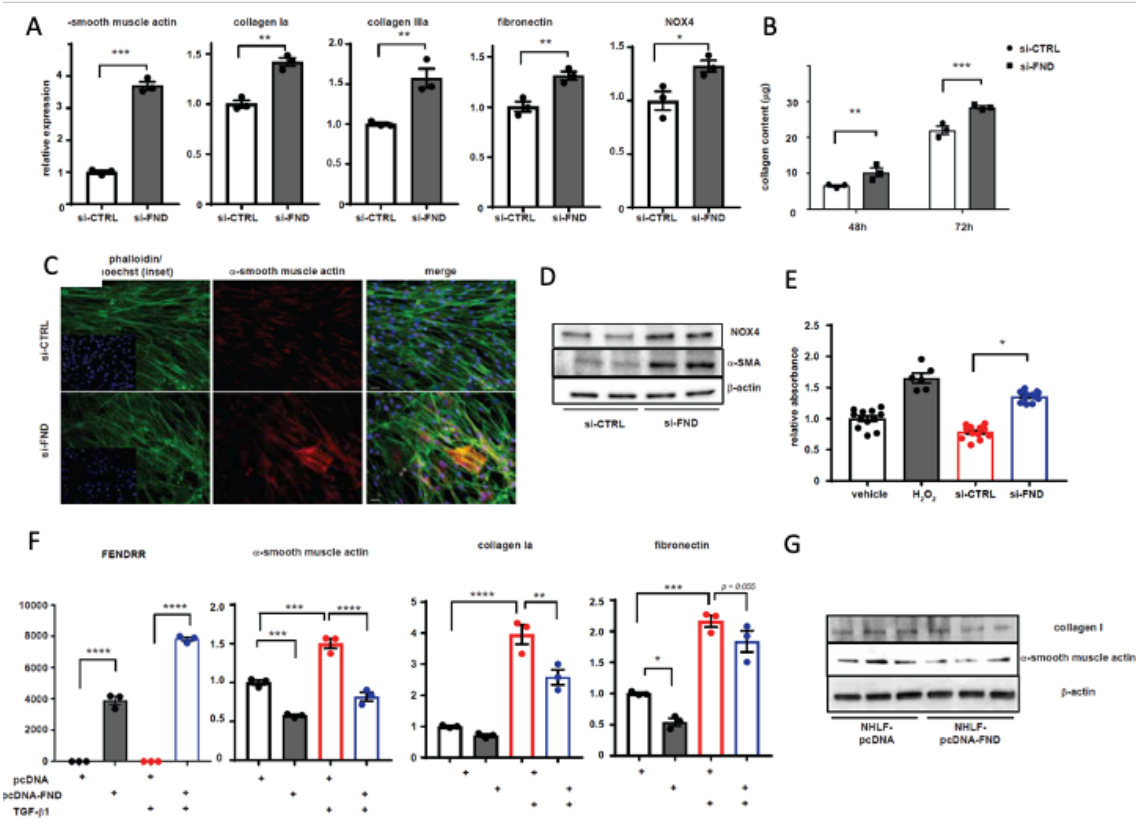


Figure 1. FENDRR effects on fibroblast phenotypes

We next aimed to assess whether replenishing FENDRR expression affects the myofibroblastic phenotypes in fibroblasts by transducing a vector expressing a full-length FENDRR transcript. As shown in **Figure 1F**, FENDRR overexpression reduced the gene expression of ACTA2, collagen Ia, and fibronectin. Western blotting confirmed the reduced expression of collagen I and ACTA2 **(Figure 1G)** Moreover, FENDRR overexpression in TGFB1 stimulated fibroblasts partially reversed the augmented gene expression of ACTA2 and collagen (**Figure 1F**).

Taken together, FENDRR suppression in human lung fibroblasts promoted differentiation into myofibroblastic phenotypes characterized by increased collagen excretion, expression of ACTA2 with stress fiber formation, with augmented NOX4 expression leading to increased ROS production.

FENDRR exerts its profibrotic effects through epigenetic regulation of the P16 and GATA6 promoters

Next, we explored the possible downstream targets of FENDRR responsible for the phenotypic regulation of fibroblasts. GATA6, one of the GATA transcriptional factors mediating cell differentiation and tissue-specific gene expression, was found as one of the most up-regulated transcriptional factors by FENDRR inhibition. GATA6 is known to regulate gene expression in smooth muscle cells. Intriguingly, previous studies demonstrated increased GATA6 expression in myofibroblasts of IPF tissue and suggested its implication in quiescence and myofibroblastic differentiation. Therefore, we proposed that induction of GATA6 mediated the phenotypic changes observed in FENDRR depleted fibroblasts. To address this proposal, we conducted co-transfection of si-RNAs against FENDRR and GATA6. Immunochemical staining confirmed GATA6 accumulated in the nucleus

of FENDRR-siRNA treated fibroblasts was successfully reversed by additional transfection of GATA6 specific si-RNAs (**Figure 2A**). GATA6 down-regulation was not shown to affect FENDRR expression levels (**Figure 2B**). We found that increased collagen Ia expression in FENDERR-siRNA treated cells was reversed by addition of GATA6-siRNA, while increased ACTA2 expression was not. (**Figure 2B**). Sircol assay confirmed that increased collagen synthesis by FENDRR knockdown was successfully reversed by inhibition of GATA6. (**Figure 2F**) In contrast, GATA6 knockdown did not result in significant reversal of augmented α-SMA protein. (**Figure 2B**)

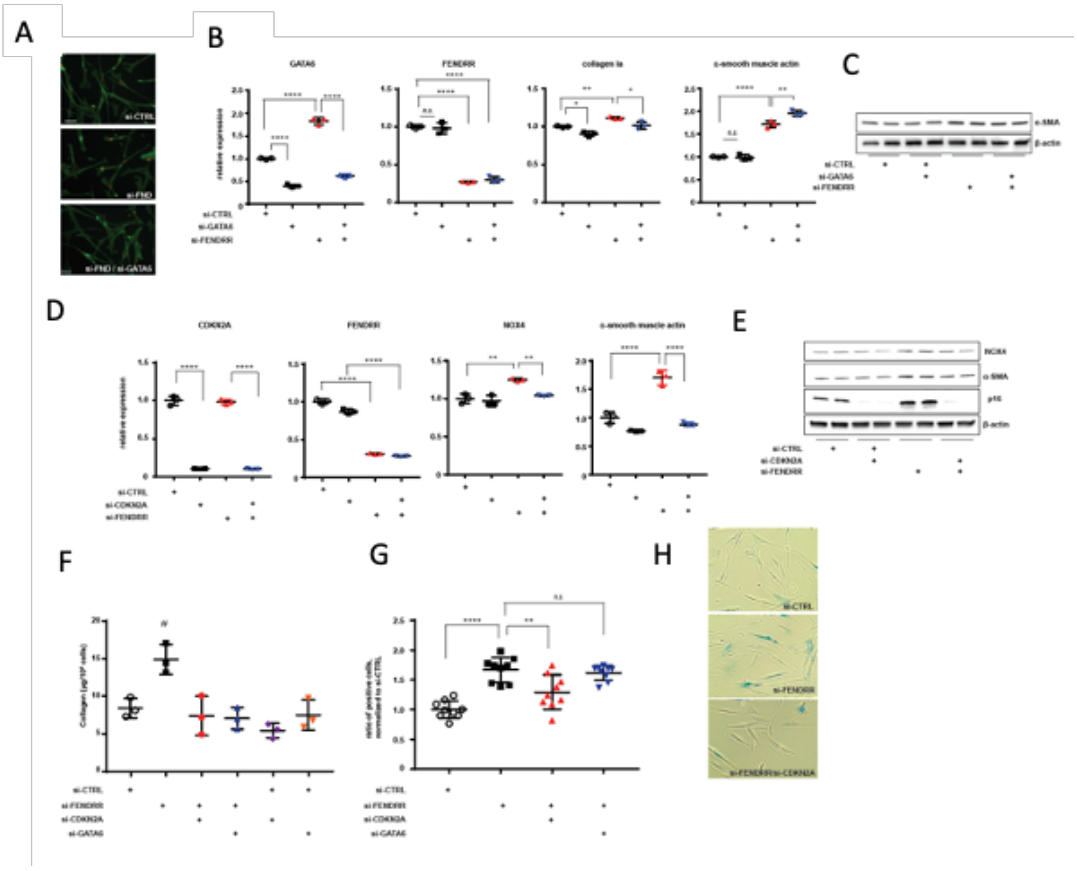


Figure 2. FENDRR effects on Collagen are mediated through GATA 6 and on ACTA2 through P16

p16^{INK4A} (encoded by CDKN2A) is one of the central regulators in cellular senescence. p16 was known as an epigenetic target of several long noncoding RNAs. In IPF, accumulation of p16INK4A protein in fibroblasts was reported. We hypothesized that p16 may be one of the down-stream targets of FENDRR and responsible for observed phenotypic changes in FENDRR depleted cells. To address this, we conducted si-RNA co-transfection with si-RNA against CDKN2A (which encodes p16^{INK4A} protein) and FENDRR. Increased expression of ACTA2 and NOX4 by FENDRR knockdown were successfully reversed by addition of p16 knockdown (**Figure 2D, E**). Further, we found collagen induction by FENDRR knockdown was reduced by co-inhibition of p16 (**Figure 2F**). As expected, increased SA-b-Gal staining in FENDRR depleted cells were recovered by addition of CDKN2A knock-down (**Figure 2G**). Taken together, our results indicated that altered fibrotic phenotypes by FENDRR inhibition in fibroblasts are, at least in part, mediated through the induction of p16 and GATA6. Given the more extensive reversal in phenotypes demonstrated by p16 knockdown, there may be a more central role for this gene in driving FENDRR mediated changes in human lung fibroblasts.

We then questioned how FENDRR modulates its downstream target genes, GATA6 and CDKN2A. FENDRR was first reported as one of hundreds of lincRNAs which interact with histone modifiers. This notion was supported by a study reporting knock-out murine model of FENDRR. We hypothesized that observed phenotypic changes in NHLFs by FENDRR inhibition were also mediated by interaction with histone modifiers, more specifically by PRC2 (polycomb repressing complex 2). PRC2 is implicated in mediating cellular senescence as well as regulating gene expression in IPF fibroblasts. Accordingly, we aimed to investigate whether FENDRR knockdown altered histone modulation on the target genes via the interaction with PRC2. We first assessed whether endogenous FENDRR was physically associated with PRC2 in human lung fibroblasts by RNA immunoprecipitation (RNA-IP) with an antibody against SUZ12, a member of the PRC2 complex, and found FENDRR was 11.6-fold enriched relative to U1snRNA negative control. This enrichment level is similar to Xist

(12.6-fold) which is a lincRNA known to be associated with the PRC2 complex (**Figure 3A**). We also found 9.3-fold association of FENDRR to EZH2, another component of PRC2 (**Figure 3B**).

We next explored whether FENDRR knockdown altered the association of PRC2 to the genomic loci of targets

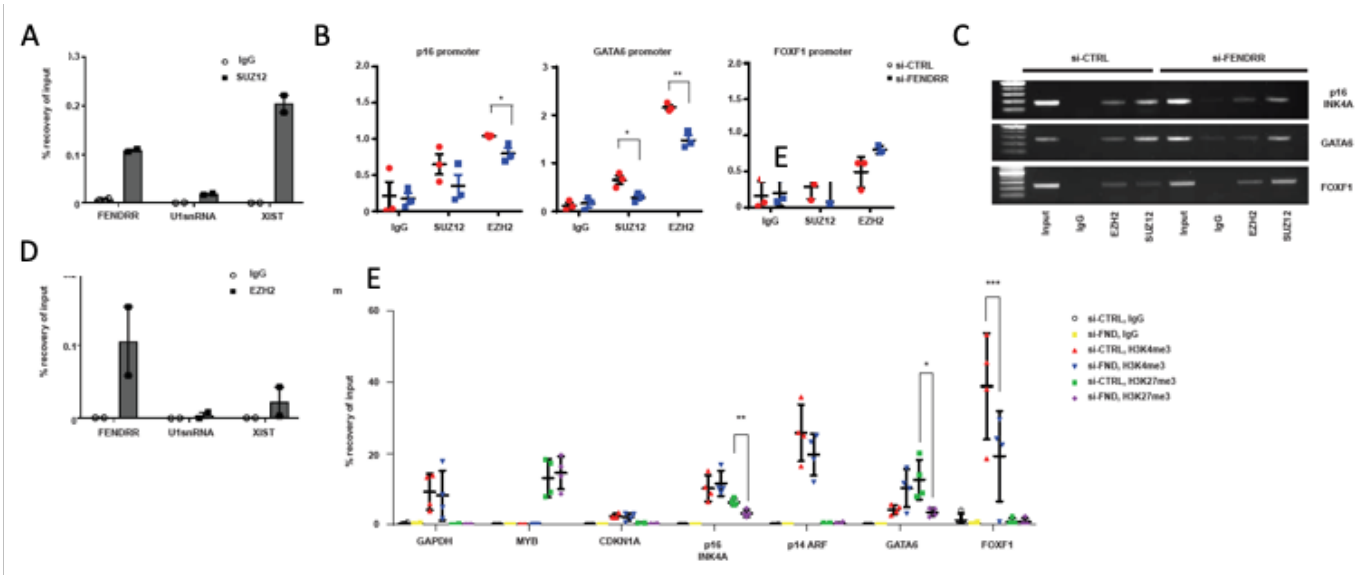


Figure 3. FENDRR mediates PRC2 association and histone methylation to the promoters of GATA6 and P16

genes (**Figure 3C**). Chromatin immunoprecipitation with SUZ12 and EZH2 antibodies suggested reduced association of PRC2 complex in the genomic region encoding GATA6 and $p16^{INK4A}$.

Finally, we assessed the histone methylation patterns on the same genomic region to ask if reduced association of PRC2 resulted in changes in the histone methylation status of the genes (**Figure 3D**). ChIP was conducted using antibodies for histone 3 lysine 27 trimethylation (H3K27me3), a repressive histone mark regulated by the PRC2 complex, and histone 3 lysine 4 trimethylation (H3K4me3), an active histone mark primarily regulated by the active histone modifier Trithorax/MLL complex. Consistent with the results of ChIP for PRC2 complex proteins, H3K27me3 mark on genomic loci on p16 and GATA6 decreased significantly in FENDRR knockdown cells, while active histone mark H3K4me3 showed a trend to increase on the same regions (**Figure 3E**). Taken together, it is proposed that FENDRR knockdown specifically lowered the recruitment of histone modifier PRC2 to target regions, thus altering the histone methylation patterns resulting in a significant decrease in repressive marks on the promoters of GATA6 and p16. This result is consistent with the increased expression of p16 and GATA6 observed in NHLFs with FENDRR knockdown.

Loss of FENDRR increased susceptibility to lung fibrosis in mice

The significance of Fendrr in development has been suggested by two previous studies using genetic knockout mice. However, they showed conflicting results. Furthermore, its roles after development or in the context of lung disease have never been explored. In bleomycin-induced lung fibrosis model, we observed reduced expression of Fendrr in fibrotic murine lungs. we aimed to elucidate the importance of FENDRR in lung fibrosis by generating a novel genetic deletion model. As shown in Figure 4A, we generated a Fendrr flox allele with the background of C57BL/6J mice using CRISPR/Cas9 technology. We bred this strain with constitutively active Cre-recombinase-positive strain to obtain mice with constitutional deletion of Fendrr genetic loci which lacks the most exons without manipulating its promoter region shared with FOXF1. We harvested lungs from a resulting mouse (actb-Cre+/-, Fendrrfl/fl; hereby we call 'mutKO' mice) and confirmed Fendrr expression was completely lost, while FOXF1 expression was not disrupted (Figure 4b). Specific deletion of Fendrr was also confirmed genome-wide transcriptome analysis in isolated lung fibroblasts (Figure 4c). We first investigated lung tissue sections to ask whether Fendrr loss affect lung architecture. We did not observe any significant architectural distortion in sections stained with hematoxylin and eosin (H&E). However, Masson's trichrome staining revealed increased deposition

of collagens in the mutKO mice lungs (Figure 4D). Sections were also studied by anti- Beta-Galactosidase staining. (Figure 4E) Increased signals were observed. Analysis of whole lung lysates exhibited increased expression in fibrotic markers including ACTA2 and collagen (Figure 4G,H), but not significant up-regulation in most molecules relevant to senescent associated signaling and secretary phenotypes, except for p21 augmentation. Next, we gave mutKO mice bleomycin intratracheally to develop lung fibrosis and assessed whether Fendrr loss affects the severity of lung fibrosis. In comparison with wildtype control, mutKO mice exhibited exaggerated infiltration of inflammatory cells and collagen deposition in lungs 14 days after bleomycin treatment (Figure 5A). mutKO mice also showed increased expression of Beta-galactosidase compared to wildtype (Figure 5B). We further confirmed that induced gene expressions (Figure 5C-E) as well as tissue deposition (Figure 5F) of collagens as measured by lung hydroxyproline that were significantly augmented in mutKO mice compared with wildtype controls. Taken together, genetic deletion of Fendrr in mice altered the susceptibility of lung fibroblasts to senescence induction, and increased severity of fibrosis by bleomycin induction.

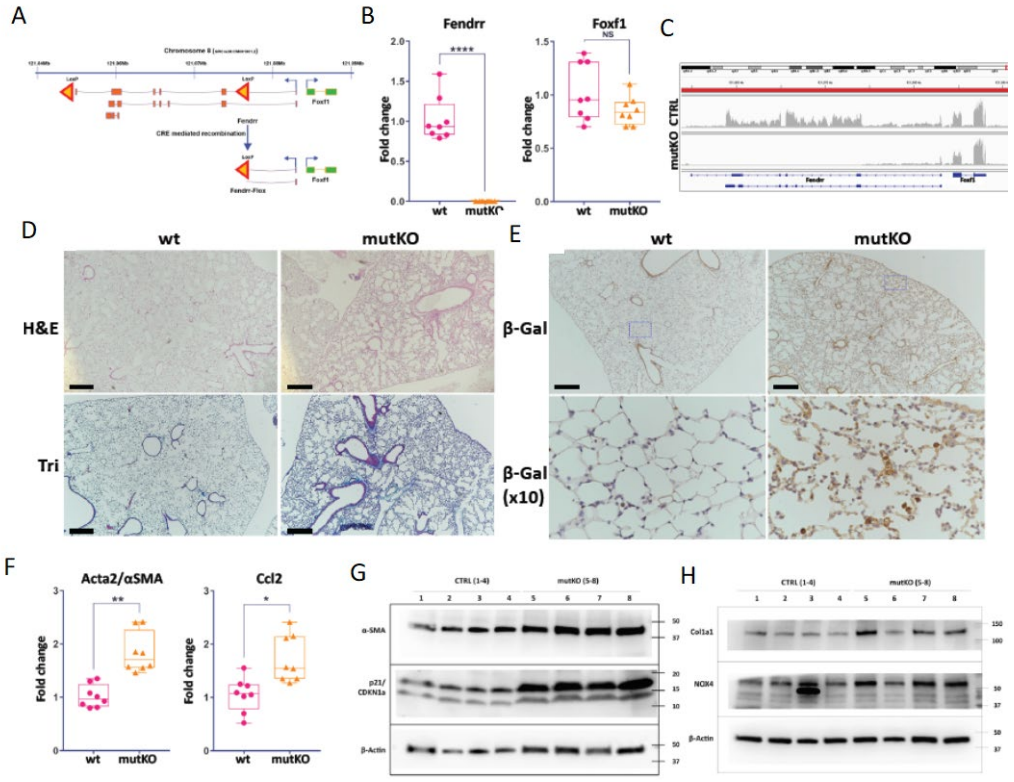


Figure 4. FENDRR deleted mice exhibit spontaneous increase in Collagen and ACTA2

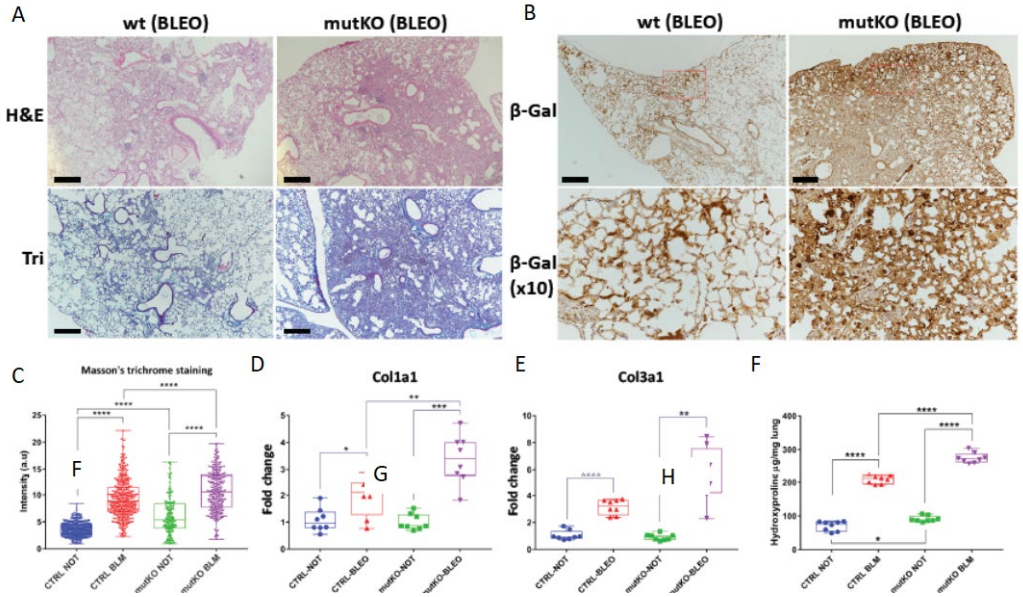


Figure 5. FENDRR deleted mice exhibit more severe fibrosis

FENDRR repletion reverses fibrosis transcriptional programs in human and mouse lung fibroblasts and reduces fibrosis in animal models of lung fibrosis.

We have designed lentiviral vectors for administration of full length FENDRR. Fibroblast cell line (Wi-38) cells were infected with either FENDRR (ectopic expression) or mock and treated with TGFB1 or vehicle. Transfection caused a dramatic increase in FENDRR levels (**Figure 6A**). TGFB1 induced increases of ACTA2 and COL1A1

were significantly blunted after FENDRR ectopic overexpression (**Figure 6B,C**). Similar effects were seen in normal human lung fibroblasts obtained from donors at different ages for COL1A1 and ACTA2 (**Figure 6D-F**). To assess whether FENDRR could have similar effects in relevance to human lung disease, we tested the effect of FENDRR supplementation in human lung fibroblasts isolated from patients with idiopathic pulmonary fibrosis. Lentiviral transfection dramatically increased FENDRR expression levels in IPF fibroblasts (Figure 6G). Infection with FENDRR caused a dramatic decrease in fibrosis markers ACTA2 (**Figure 6H**) and COL1A1 (**Figure 6I**). To study the effects of FENDRR augmentation in animal models of disease, we isolated primary mouse lung fibroblasts from lung tissues (CTRL: Fendrr flox+/+ mice, mutKO: actCre+/+ Fendrr loxp+/+ mice). Fibroblasts were cultured in DMEM/F12 with 15% FBS under hypoxia setting (3% O2) and evaluated more than 2 weeks after isolation in this study. Fendrr sequences were cloned into pIRES-EGFP vector. Isolated mouse primary fibroblasts were transfected with pIRES-EGFP-Blank or pIRES-EGFP-Fendrr. 36h post-transfection, fibroblasts were cultured and stimulated by TGFβ (5ng/ml) or PBS for 48h. Cells were analyzed by qRT-PCR and western blot. pIRES-EGFP-Fendrr caused dramatic increases in FENDRR expression (**Figure 7A**). Infection with FENDRR caused a reduction in ACTA2 (**Figure 7B**) and COL1A1 (**Figure 7C**) at baseline and in response to TGFB1 in both wildtype and mutKO mice. This effect was also seen at the protein level (**Figure 7D**). Administration of lentiviral FENDRR to mice blunted bleomycin induced fibrosis as reflected by lung hydroxyproline (**Figure 8A**) and mason trichrome staining (**Figure 8B**), but changes in gene expression by qRT PCR did not reach statistical significance. Taken together, our results indicate that supplementation of FENDRR is feasible and may have potential antifibrotic effects and thus should be considered for development of therapeutics.

Figure 6: Effect of FENDRR on profibrotic properties of a fibroblast cell line (Wi-38), normal human lung fibroblasts (NHLF) and IPF fibroblasts (IPF-LF)

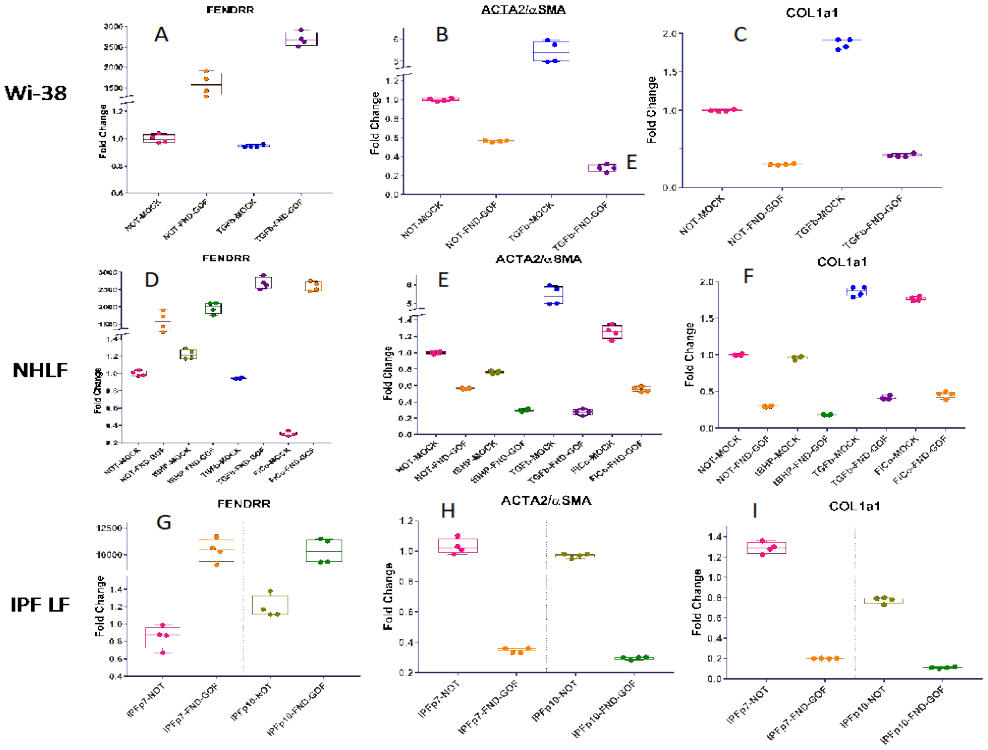
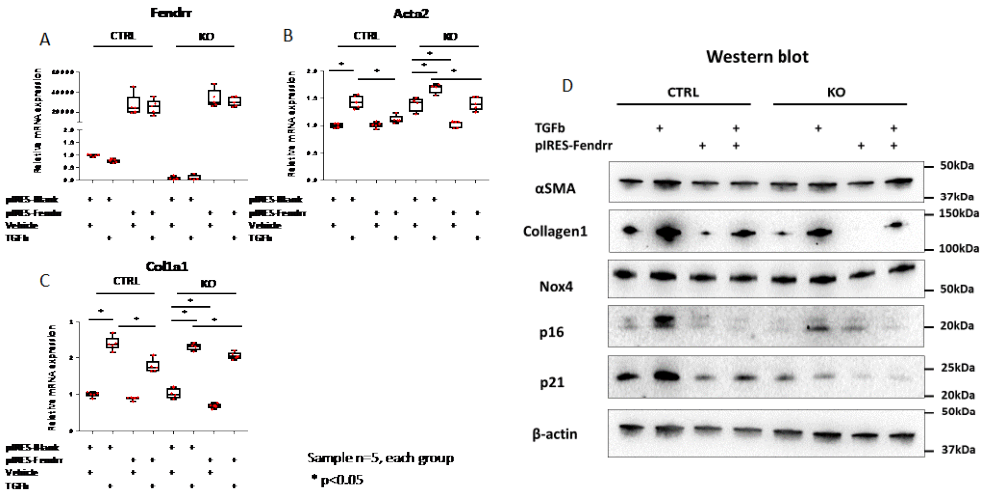


Figure 7: FENDRR supplementation reverses profibrotic properties in mouse lung fibroblasts and reverses phenotype of mutKO fibroblasts



FENDRR is specifically downregulated in the human IPF Lung

Using updated annotation databases (Ensemble 81 gene and Broad Institute), we re-annotated probes and obtained expression datasets comprising of 412 human lung samples with 3829 lincRNA probes (**Figure 9A**). Expression profiles of lincRNAs among different lung diseases demonstrated that lungs with IPF showed clear differences relative to normal controls and COPD lungs (**Figure 9B**). We identified 1497 (39.1%) differentially expressed probes between IPF lungs and normal controls (FDR <0.05). Similarly, 1677 (43.8%) probes were different between IPF lungs and COPD lungs. In contrast, only 276 (7.2%) probes were found to be differentially expressed in the comparison between control and COPD, which suggests a more distinct and widespread lincRNA expression disturbance in IPF pathology relative to COPD. Focusing on probes with more than 2-fold difference, we identified 31 probes at the intersection of probes down-regulated in IPF relative to controls and IPF relative to COPD. Intriguingly, 8 out of these 31 probes corresponded to the transcripts from a single lincRNA gene, FENDRR (FOXF1 Adjacent Non-Coding Developmental Regulatory RNA). (**Figure 9C**) We validated our initial findings of microarray using the nCounter system which captures and counts individual transcripts with high sensitivity and without enzymatic reactions or amplification bias. We assessed the expression of the 37 lincRNA probes which were most differentially expressed in the microarray data. Expression data obtained from microarray and nCounter were well correlated, validating our initial findings. (**Figure 9D**). nCounter results confirmed the significant down regulation of FENDRR transcripts in IPF lungs against control lungs and COPD lungs but also demonstrated that FENDRR decrease was specific to IPF and was less prominent in CTD-ILD or NSIP (**Figure 9E**). Confirmation of the results using data from RNA sequencing of 23 samples from IPF lungs compared to COPD lungs (n = 44) and control lungs (n = 22) also demonstrated decreased FENDRR expression relative to COPD and normal control (**Figure 9F,G**). Correlation of FENDRR with clinical parameters did not reveal any significant association with PFT potentially because the vast majority of samples were obtained from patients with advanced disease. Taken together, we have demonstrated that FENDRR is decreased in human IPF lungs and that this decrease is specific to IPF compared to other advanced lung disease and replicated using different technologies.

Figure 8: Lentiviral FENDRR supplementation reverses blunts bleomycin induced fibrosis. (NN - Control PBS, BN – Bleomycin PBS, BG – Lenti blank, BLFp – viral titer 10^7 , BLFi – viral titer 10^5.

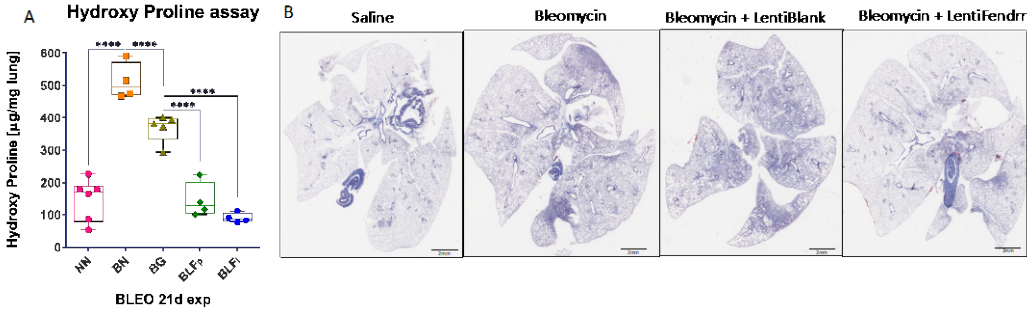
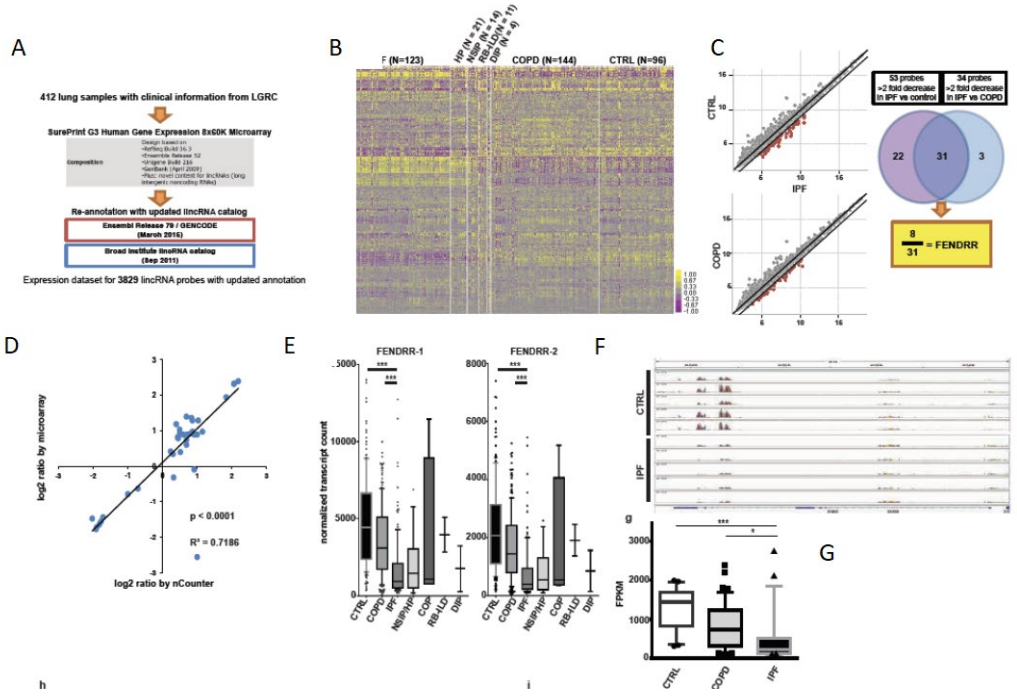


Figure 9: FENDRR is specifically decreased in the human IPF lung



In summary overall, we have accomplished the tasks of our project. We demonstrated that FENDRR expression maintains fibroblasts differentiation status through its effects on chromatic organization and demonstrated that FENDRR expression is required to prevent fibrosis. We identified the epigenetic mechanisms by which FENDRR affects fibroblast phenotypes and supported our results by both loss and gain of function experiments, established a role for FENDRR in-vivo in animal models of pulmonary fibrosis and confirmed the implications of FENDRR downregulation in human lungs with Pulmonary Fibrosis**. Taken together, we have established the role of FENDRR as a key regulator of pulmonary fibrosis with potential therapeutic implications.**

- **What opportunities for training and professional development has the project provided?**
  - *"Nothing to Report."*
- **How were the results disseminated to communities of interest?**
  - *"Nothing to Report."*
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - *"Nothing to Report."*

1. **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:*
   - **What was the impact on the development of the principal discipline(s) of the project?**
     - *Nothing to report*
   - **What was the impact on other disciplines?**
     - *Nothing to report*
   - **What was the impact on technology transfer?**
     - *Nothing to report*
   - **What was the impact on society beyond science and technology?**
     - *Nothing to report*
2. **CHANGES/PROBLEMS:** *None*
   - **Changes in approach and reasons for change**
   - **Actual or anticipated problems or delays and actions or plans to resolve them**
   - **Changes that had a significant impact on expenditures, vertebrate animals, biohazards, and/or select agents**
   - **Significant changes in use or care of vertebrate animals.**
   - **Significant changes in use of biohazards and/or select agents**
3. **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."*
   - **Publications, conference papers, and presentations**
     Report only the major publication(s) resulting from the work under this award.
     - **Journal publications.** *A publication is now being put together*
     - **Books or other non-periodical, one-time publications.** *None*

- **Conference Presentations**
- **Dr. Kaminski:**
    - 2019 Pulmonary Seminar Series, University of Pennsylvania, Penn Cardiovascular Institute & Penn Center for Pulmonary Biology, Philadelphia, PA. "Pulmonary Fibrosis – an RNA centered perspective"
    - 2018: Pulmonary Grand Rounds, New York University Langone Health, New York, NY. "Pulmonary Fibrosis – Lessons from RNA"
    - 2018: Training Grant Retreat, Columbia University Medical Center, New York, NY, "Sorting Through the Kitchen Sink – Understanding Pulmonary Fibrosis Using RNA"
    - 2018: American Thoracic Society International Conference, The Gene-Environment Interaction in Interstitial Lung Disease, Washington, DC, "The Environment, Epigenetics, Non-Coding RNAs and Interstitial Lung Disease"
    - 2017: American Thoracic Society International Conference, Innovative Clinical Trials in Pulmonary Fibrosis and Beyond, Washington, DC, "When a Genomics Expert Tries to Develop a Drug: Lessons from the NHLBI CADET Program"
    - 2017: Discovery Series Lecturer, The Ohio State University, Columbus, Ohio, "Pulmonary Fibrosis – new biomarkers & role of non-coding RNAs"
    - 2017: Neff Lecturer, Department of Medicine Grand Rounds, University of Colorado, Denver, Colorado, "Idiopathic Pulmonary Fibrosis – how RNA profiling led to validated biomarkers and novel therapies'
    - 2017: Excellence in Respiratory Medicine, University of Colorado, Denver, Colorado, "It all connects – from non-coding RNAs to mitochondrial disfunction in IPF"
    - 2016: University College London, London, United Kingdom, "Normalizing cellular phenotypes in Pulmonary Fibrosis – from non-coding RNAs to mitochondrial homeostasis"
    - 2016: Pulmonary Research Conference, Yale University School of Medicine, New Haven, Connecticut, "FENDRR lincRNA at the crossroads of aging and fibrosis
- Dr. Sakamoto
    - 2018 Sakamoto K. "Epigenomics, including non-coding RNA" 58th Annual Meeting of the Japanese Respiratory Society 2018, Osaka, Japan. Apr 2018 (Invited talk in Young Investigator Symposium)
    - 2017 Sakamoto K. "LncRNA Regulation of Fibroblast Transcriptional Networks in Pulmonary Fibrosis" Gordon Research Conference, Lung Development, Injury & Repair 2017, New London, NH. Aug 2017 (Invited Lecture)
    - 2017 Sakamoto K. "Decreased expression of FENDRR, a lung mesenchymal long non-coding RNA, regulates fibroblast phenotypes in IPF through NOX4" ERS International Congress 2017 Milan, Italy, Sep 2017 (Oral presentation)
    - 2016 Sakamoto K. "FENDRR is an Epigenetic Regulator of Cellular Senescence in Pulmonary Fibroblasts" American Thoracic Society 2016 International Conference, San Francisco, CA, May 2016. (Orally presented in Scientific Breakthrough Session)

Published Conference Abstracts

- Decreased expression of FENDRR, a lung mesenchymal long non-coding RNA, regulates fibroblast phenotypes in IPF through NOX4 Koji Sakamoto, Nikos Xylourgidis, Norihito Omote, Taylor Adams, Guoying Yu, Farida Ahangari, Jose Herazo-Maya, Naftali Kaminski, Robert Homer European Respiratory Journal 2017 50: OA2909; DOI: 10.1183/1393003.congress-2017.OA2909
- Loss of lncRNA FENDRR Induces Senescence in Adult Mouse Lungs. N. Xylourgidis, K. Sakamoto, J.C. Schupp, T. Adams, G. DeIuliis, N. Omote, N. Hashimoto, Y. Hasegawa, N. Kaminski. May 2019 C59. GENETIC AND EPIGENETIC MECHANISMS IN PULMONARY FIBROSIS
- In-Vivo Deletion of FENDRR, A Large Non-Coding RNA, Induces Spontaneous Fibrotic Changes in Knockout Mouse Lungs. N. Xylourgidis, K. Sakamoto, N. Aurelien, N. Kaminski. May 2018 A71. THE EPIGENOME, GENOME AND NON-CODING RNAs IN LUNG DISEASE
- Single Cell RNA-Sequencing Reveals Distinct Effects of Inhibition of FENDRR, a Long Non-Coding RNA Implicated in Fibroblast to Myofibroblast Differentiation. Taylor Adams, Koji Sakamoto, Farida Ahangari, Azim Munivar, Naftali Kaminski. May 2017 B97. FLIPPING THE SWITCH: DETERMINANTS OF FIBROSIS
- FENDRR Is an Epigenetic Regulator of Cellular Senescence in Pulmonary Fibroblasts. Koji Sakamoto, Brenda Juan Guardela, Guoying Yu, Jose Herazo-Maya, Farida Ahangari, Argyrios E. Tzouvelekis, Robert Homer, Naftali Kaminski. May 2016 B62. THE BIOLOGY OF SCARRING. WHERE ARE WE NOW

- **Internet site(s)**
  None
- **Technologies or techniques**
  None
- **Inventions, patent applications, and/or licenses**
  None
- **Other Products**
  We generated a Fendrr flox allele with the background of C57BL/6J mice using CRISPR/Cas9 technology. We bred this strain with constitutively active Cre-recombinase-positive strain to obtain mice with constitutional deletion of Fendrr genetic loci which lacks the most exons without manipulating its promoter region shared with FOXF1

4. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

None

- **What individuals have worked on the project?**
  - *Provide the following information for: (1) PDs/PIs; 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). If information is unchanged from a previous submission, provide the name only and*

indicate "no change."

Name	*Naftali Kaminski*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*eBRAP ID: Kaminx*
Nearest person month worked:	*2*
Contribution to Project:	*Dr. Kaminski is the principal investigator of this project and in charge of all aspects related to design and execution of all of the aims in the project.*
Funding Support:	*N/A*

Name	*Robert Homer*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Homer performed and oversaw the histologic evaluations of mouse lungs in this grant.*
Funding Support:	*N/A*

Name	*Patty Lee*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Lee helped in all lenti-viral and transgenic experiments.*
Funding Support:	*N/A*

Name	*Norihito Omote*
Project Role:	*Postdoctoral Associate*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Omote performed cell culture and animal experiments as needed.*
Funding Support:	*N/A*

Name	Nikos Xylourgidis
Project Role:	*Associate Research Scientist*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*12*
Contribution to Project:	*Dr. Xylourgidis led all research activities, mouse experimentation and breeding strategy, cell culture and mechanistic studies.*
Funding Support:	*N/A*

Name	Joe DeIuliis
Project Role:	*Laboratory Manager*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Mr. DeIuliis is in charge of all orders requisitions, and supervises all of the needed orders for this project.*
Funding Support:	*N/A*

- **Has there been a change in the active other support of the PD/PI(s) 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.*

These are the changes in the Active Other Support since September 2018 for the PI, Dr. Kaminski.

New Grants:

R01 HL141852 (Kaminski) 12/01/18 – 11/30/22 1.8 calendar months
NIH/NHLBI $537,822 / annual direct
Epithelial Protective Effects of Thyroid Hormone Signaling in Fibrosis
In this proposal we aim to use thyroid hormone signaling to improve mitochondrial function and enhance epithelial cell survival as an antifibrotic strategy. Role: PI

(Diversity Supplement)
R01 HL141852-02S1 (Kaminski) 02/01/20 – 11/30/22 0.12 calendar months
NIH/NHLBI $132,802 / annual direct
Epithelial Protective Effects of Thyroid Hormone Signaling in Fibrosis
In this supplement to support Postgraduate Associate Kadi-Ann Rose, we hypothesize that augmentation of thyroid hormone signaling affects the profibrotic phenotype of IPF fibroblasts through its effects on TGFB1 signaling and cellular metabolic pathways. Role: Mentor

U01 HL145567 (Kaminski) 01/01/19 – 12/31/22 1.2 calendar months
NIH/NHLBI $506,406 / annual direct
Normal Aging Lung Cell Atlas (NALCA)
The overall objective of the Normal Aging Lung Cell Atlas project is to identify cell and microenvironment specific molecular regulatory events that happen during normative lung aging and use them to develop novel biomarkers and therapeutics. We will share those with the scientific community, through a highly interactive and intuitive web interface called AgeDREMM: the aging lung dynamic regulatory multicellular model. Role: PI

1 R01 HL152677-01 (Herzog) 04/01/20 – 03/31/26 0.36 calendar months
NIH/NHLBI $362,777 / annual direct
Macrophage driven, profibrotic adrenergic nerve remodeling in SSc-ILD
The goal of this project is to understand the mechanism through which macrophage derived netrin 1 guides post injury nerve remodeling using animal models and samples from patients with SSc-ILD. Role: Co-I

PR181442 (Dela Cruz) 09/01/19 – 08/31/22 0.36 calendar months
Department of the Army $391,440 / annual direct
IL-15-Mediated IL-6Ra Effector Memory CD8+ T cells in Dysfunctional Lung Responses During Cigarette Smoke Exposure and Influenza Viral Infections
This grant will investigate the cellular and molecular mechanisms by which viruses interact with CS to regulate injury and fibrotic responses in the lung. Role: Co-I

PR182416 (Schupp) 03/01/19 – 08/31/20 0.3 calendar months
Department of Defense, PRMRP Discovery Award $132,565 / annual direct
Identifying Reversible Molecular Networks in Human Pulmonary Fibrosis using Single Nuclear Transcriptomics and Systems Biology
This grant will fund a systems biology approach to substantially impact our understanding of pulmonary fibrosis and its disease progression and to discover cell type-specific candidates for novel therapeutics for patients suffering from PF. The proposal project is highly innovative and potentially transformative, and we are confident that it will bring advances to ultimately cure PF. Role: Co-I

R01 AR074997 (Varga, J) 06/13/19 – 04/30/24 0.36 calendar months
Northwestern University (NIH) $75,000 / annual direct
Damage-Associated Molecular Patterns Driving Fibrosis Progression in Scleroderma
The Yale team will be responsible for the execution of Aim 3. Specifically, Yale will perform all aspects of Aim 3 including the single cell RNA Seq and its correlation to the *ex vivo* response of skin and lung fibroblasts to fibrogenic stimuli. Role: Co-I

Northwestern University (Whitsett) 07/01/19 – 06/30/22 0.096 calendar months
HCA Lung Seed Network $13,044 / annual direct
Dr. Kaminski, along with Dr. Whitsett, will be responsible for the Tier 3 computational groups, which will provide a platform for data collection, processing, analysis and integration into a functional lung atlas. Role: Site PI

Veracyte (Kaminski) 04/20/20 – 11/30/20 0.7 calendar months
$108,898
Using scRNAseq to identify disease specific immune cellular aberrations in chronic fibrosing lung disease
The overall objective of this pilot proposal is to apply the powerful technology of single cell profiling to identify immune aberrations in the peripheral blood that distinguish IPF from exposure related CHP and autoimmune CTD-ILD and to use them to guide therapy. Role: PI

These grants have closed since the last report in September 2018
R01 HL127349 (Kaminski) 8/14/15 – 5/31/20 (NCE) 2.4 calendar months
NIH/NHLBI $598,807 / annual direct
Genomic Analysis of Tissue and Cellular Heterogeneity in IPF
Idiopathic Pulmonary Fibrosis (IPF), a chronic progressive lung disease that affects more than 5 million people worldwide, has a 2-5 year median survival without transplantation and currently has no effective therapy. The ability to decipher the molecular mechanisms that characterize or cause IPF is a critical step towards developing effective therapeutic strategies. Using human IPF lungs, advanced genomic technologies, and computational and analytical methods, we will identify the key regulatory molecular and genetic events that determine the progression of IPF to better understand the disease and to design novel therapeutic interventions in this devastating disease. Role: PI

R01 HL125850-01A1 (Herzog) 4/1/16 – 3/31/20 0.36 calendar months
NIH/NHLBI $250,000 / annual direct
Neuroimmune Molecules in Scleroderma Lung Fibrosis
Goals: Evaluate the contribution of neuroimmune molecules to the pathogenesis of scleroderma related lung fibrosis. Role: Co-I

2 R01 HL109233-06A1 (Herzog) 8/3/11 – 4/30/20 0.6 calendar months
NIH/ NHLBI $256,511 / annual direct
Neuronally Active Proteins in IPF
The aim of the proposed study is to elucidate the contribution of neuronally active proteins to the aberrant repair responses that characterize Idiopathic Pulmonary Fibrosis (IPF). Role: Co-I

U54 HG008540 (Cooper / University of Pittsburgh) 9/29/14 – 8/31/19 0.5 calendar months
NIH NHLBI $65,322 / annual direct

Center for Causal Modeling and Discovery of Biomedical Knowledge from Big Data

In this grant, we propose to capitalize on the publicly available and private data from the Lung Genomics Research Consortium (LGRC) and Lung Tissue Research Consortium (LTRC) resources to develop the software and tools to identify causal relationships between (1) omic data and image features and (2) disease characteristics and subtypes. Role: Subcontract PI

U01 HL122626 (Kaminski) 6/15/14 – 4/30/20 1.0 calendar months
NIH / NHLBI $136,668 / annual direct

Alveolar DevMAP

The overall objective of "Alveolar DevMAP" is to generate a compendium of the dynamic and regional changes in epigenetic marks, microRNA, mRNA and proteins that happen during alveolar septation and use this compendium to generate a dynamic temporal regulatory model of normal alveolar septation. To address this objective, we have assembled a multidisciplinary group of experts in lung development, genomics, epigenomics, quantitative imaging, systems and computational biology, and biostatistics. Role: PI

1 UH2 HL 123876-01 (Chupp/Elias) 9/22/14 – 6/30/19 0.6 calendar months
NIH/NHLBI $1,236,650 / annual direct

Preclinical Development of a Novel Anti-YKL-40 Biologic to Treat Severe Asthma

We will complete the pre-clinical development of a humanized monoclonal antibody against the chitinase-like protein, YKL-40, for the treatment of severe asthma. A companion diagnostic test will also be developed to measure YKL-40 in the serum that is drug-bound or free for monitoring the bioavailability, dosing, and biologic effect. The results will generate a novel therapeutic against a validated mediator of inflammation and remodeling in asthma and will lead to an Investigational New Drug (IND) application following the conclusion of the award. Role: Co-I

- **What other organizations were involved as partners?**
  - *Nothing to report*

5. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:**
   - **QUAD CHARTS:**
6. **APPENDICES:**