# Secreted proteins from pluripotent stem cells improve the function of AT2 cells undergoing fibrotic stress

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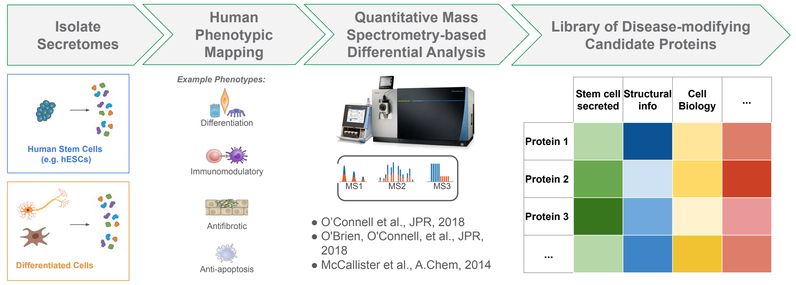
## Introduction

- Juvena is developing drugs that target AT2 cell regeneration under chronic fibrotic stress.
- Screening factors from our library of secreted proteins produced by pluripotent stem cells identified factors that promote AT2 cell viability, cell function, and reduce EMT in human in vitro models of pulmonary fibrosis.
- These factors show potential for improving lung function through promoting AT2 renewal and inhibiting fibrosis in mouse models of fibrosis.

## Methods




- O'Connell et al., JPR, 2018
- O'Brien, O'Connell, et al., JPR, 2018
- McCallister et al., A.Chem, 2014

**Figure 1:** Schematic of Juvena Therapeutics, Inc proprietary regenerative protein library generation pipeline. Secretomes from pluripotent stems cells are isolated and mapped for disease-modifying effects through in vitro phenotype mapping. Quantitative mass spectrometry is used to determine the individual protein composition and abundance of each secretome. Proteins with pro-regenerative properties are selected and ranked using bioinformatics analysis and machine learning models trained on a proprietary feature set comprised of multi-dimensional map of proteins and their diseases-modifying effects across different organs and indications. Features include chemistry properties, sequence information, evolutionary parameters, cell biology and protein-to-protein interaction information. IP protection (CoM and Method of Use). Our approach to screening human stem-cell secreted proteins (patent no.: 10821155) has so far generated a library of ~1,000 bioactive human signaling proteins ("rJUV-1k") identified from human embryonic stem cells. We are expanding on the library by screening multiple additional multipotent and totipotent stem cell secretomes.

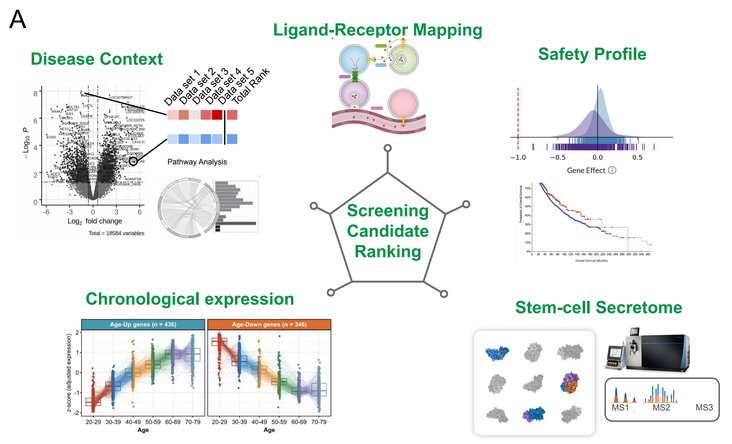



**Figure 2:** Schematic of Juvena Therapeutics, Inc proprietary candidate ranking pipeline. Juvena's artificial intelligence-enabled platform predicts and rank orders therapeutic signaling proteins from a proprietary disease-modifying secreted protein library to screen and generate hits, followed by target validation, lead selection, and lead optimization to generate a growing pipeline of regenerative biologics for chronic and age-related diseases. (A) The applications of quantitative proteomics, transcriptomics, and computer vision with machine learning feeds high dimensional, multimodal data into a compounding database enabled by our growing human therapeutic protein library to hasten the process of systematic biologics drug discovery and development (1 issued and 13 pending patents to date).

## Results

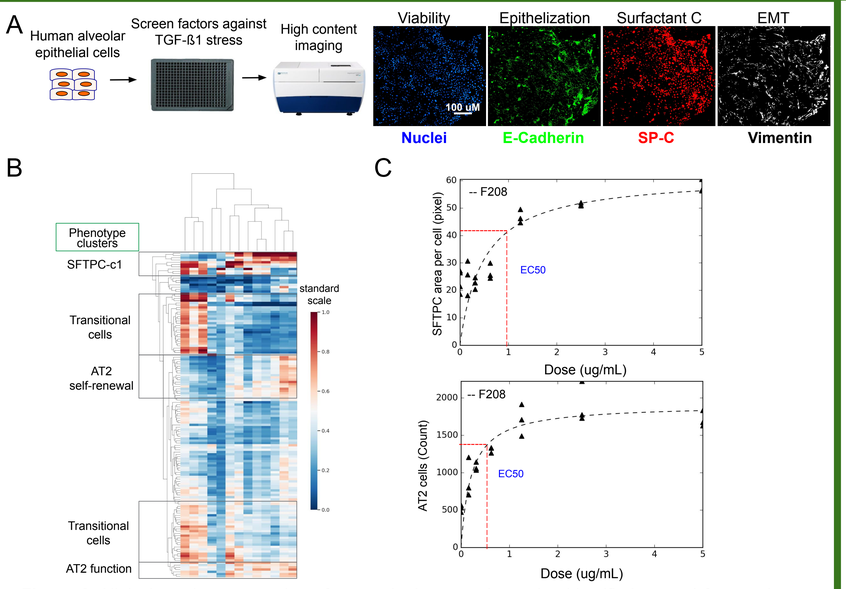



**Figure 3**: High throughput screening of pre-ranked secreted proteins identified several factors that promote AT2 regeneration. (A) Schematic of the screening pipeline to identify factors that promote AT2 function under fibrotic stress. (B) Heatmap clustering of factors that alter AT2 phenotypes. Cells: Human AT2 cells. Media: Basal media (BM) (n=3 human donor cells). (C) Representative dose range curves of factors that improve surfactant C expression and increase AT2 cell counts.

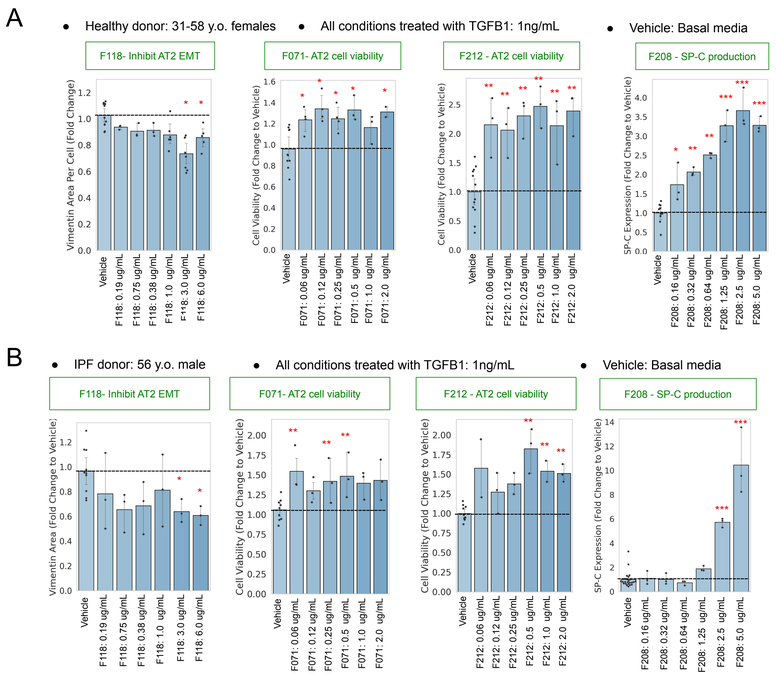



**Figure 4**: Secreted factors promote AT2 function and inhibit EMT under fibrotic conditions in healthy and IPF AT2 cells (A) Schematic of the screening pipeline to identify factors that promote AT2 function under fibrotic stress. (B) Heatmap clustering of factors that alter AT2 phenotypes. Cells: Human AT2 cells. Media: Basal media (BM) (n=3 human donor cells). (C) Representative dose range curves of factors that improve surfactant C expression and increase AT2 cell counts.

## Results

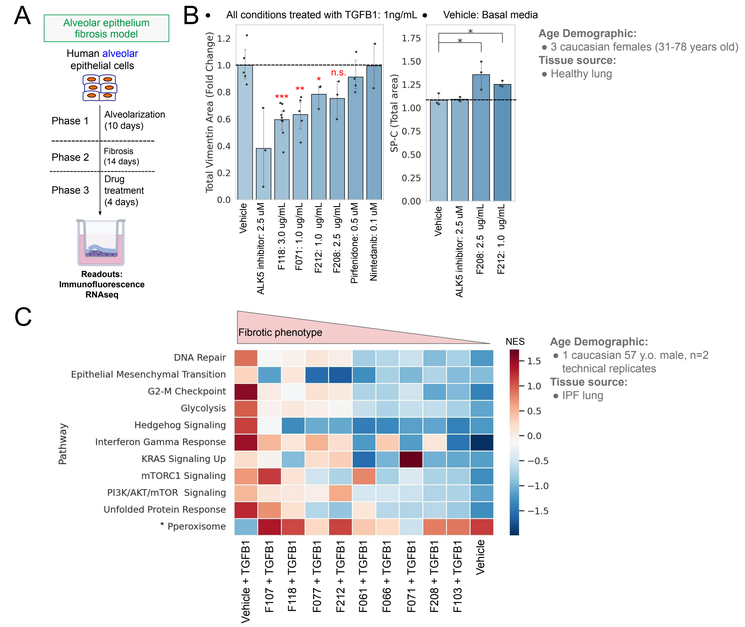



**Figure 5**: Secreted factors promote AT2 function and inhibit EMT under fibrotic conditions in an air-liquid-interface (ALI) model of chronic fibrosis (A) Schematic of the ALI based fibrosis model. (B) EMT phenotype and SP-C production of human AT2 cells under fibrotic stress (n=1-3 biological replicates). (C) Heatmap of fibrotic and cellular stress pathways upregulated by TGFB1 treatment are attenuated by multiple secreted proteins. *Pperoxisome is indicative of lipid metabolism and is associated with surfactant production

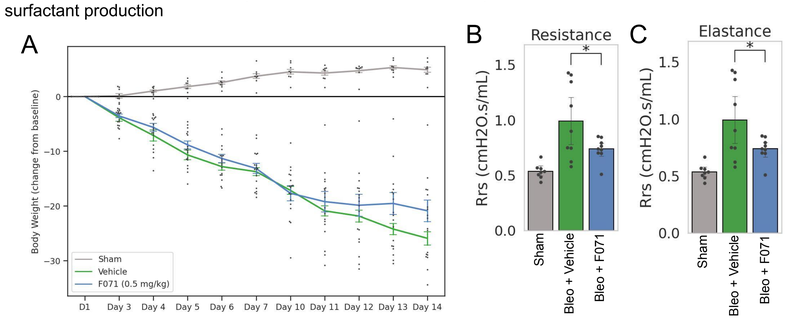



**Figure 6**: Secreted factors improve lung function in a bleomycin model of IPF (A) Line plot of weight change in mice across the study (n=10). 12 week old male C57Bl/6 mice were injected with 1.3 mg/kg bleomycin. Vehicle or F071 was injected I.P. daily starting day 7 post-bleomycin. (B) Bar graph of lung resistance (airway constriction) (n=8-10). (C) Bar graph of lung elastance (lung stiffness) (n=8-10).

## Conclusions

1. Secreted proteins from Juvena's proprietary library were pre-ranked using a suite of artificial intelligence tools for *in vitro* IPF phenotype screening.
2. Multiple factors promoted human AT2 cell activity under fibrotic stress by multiple mechanisms including reducing EMT and improving SP-C production.
3. Pathway analysis of RNAseq data elucidated the MOA for different factors in ameliorating fibrosis and promoting alveolar regeneration in AT2 cells from IPF lung.
4. Promising lung function improvement in animal studies is seen by body weight and lung function parameters.

**Acknowledgements**

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**References**

O'Connell JD, Paulo JA, O'Brien JJ, Gygi SP. Proteome-Wide Evaluation of Two Common Protein Quantification Methods. J Proteome Res. 2018;17(5):1934–1942. doi:10.1021/acs.jproteome.8b00016

Yousef, H., Conboy, M. J., Li, J., Zeiderman, M.,Vazin,T., Schlesinger, C., Conboy, I. M.(2013). hESC-secreted proteins can be enriched for multiple regenerative therapies by heparin-binding. Aging. https://- doi.org/10.18632/aging.100559

Yousef, H., Conboy, M. J., Mamiya, H., Zeiderman, M., Schlesinger, C., Schafer, D.V., & Conboy, I. M.(2014). Mechanisms of action of hESC-secreted proteins that enhance human and mouse myogenesis. Aging, 6(8), 602–620. https://doi.org/10.18632/aging.100659

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