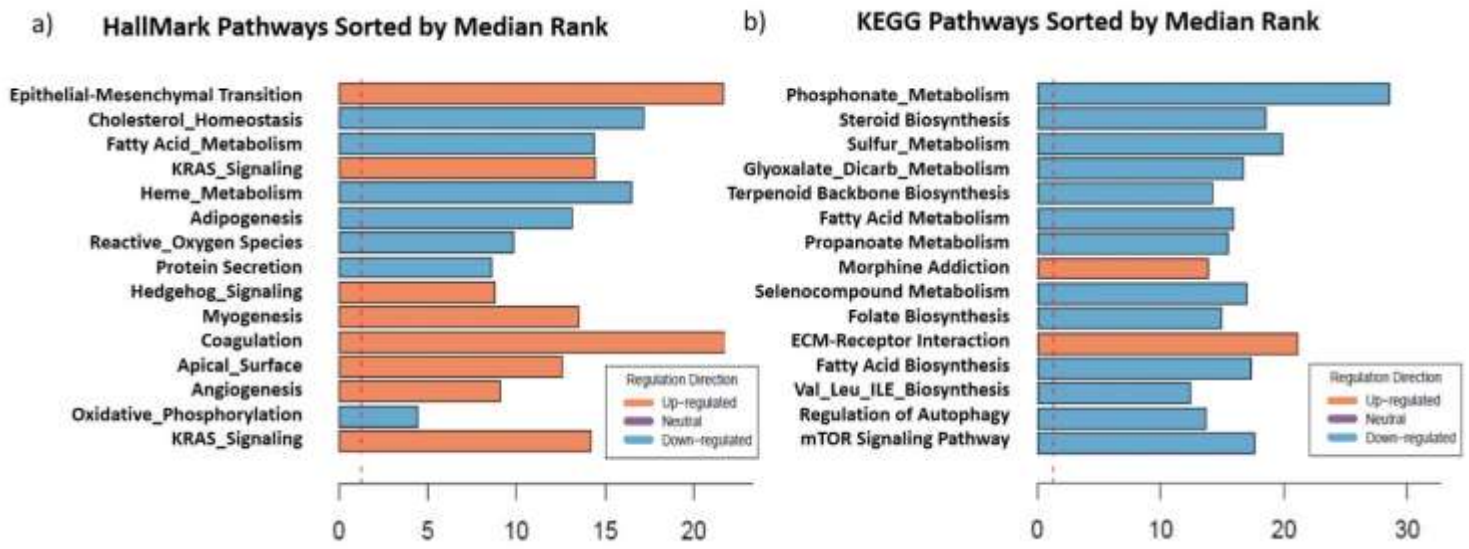
Supplementary Material

Materials and Methods

Human Subjects and Lung Tissue Acquisition: All human subject sample acquisitions and experiments were conducted with the appropriate approval from the Institutional Review Board (IRB 806468, IRB 813685). The clinical profile and demographics of IPF, ALI and control subjects are listed in Table 1. The IPF cohort consisted of 36 subjects with advanced IPF (mean % predicted FVC 44) that underwent lung transplantation at the University of Pennsylvania. The ALI and control cohorts consisted of subjects whose donated lungs were deemed ineligible for lung transplantation. Explant samples were evaluated by an experienced thoracic pathologist who classified samples as 'ALI' based on the presence of diffuse alveolar damage or as 'control' if no abnormal pathology was present. Explanted tissue samples was stored in RNAlater at -80℃.

RNA Isolation: ~5mg of lung tissue was agitated with a 5mm stainless steel bead in Qiazol lysis solution (Qiagen Inc) using a TissuelyserII followed by extraction with chloroform. Isolated total RNA was precipitated with 70% ethanol and purified using the RNeasy mini kit (Qiagen Inc) following the manufacturer's protocol. RNA concentration, quality and integrity was assessed by Nanodrop and Bioanalyzer (Agilent Technologies). All samples yielded high quality RNA.

Supplementary Figure S1. Enhanced Gene Set Enrichment Analysis (EGSA) of differential gene regulation in IPF lung. Gene lists were generated using a cutoff of positive and negative fold change ≥1.5 and adjusted P value of ≤ 0.1 and analyzed with the "msigdb" data analyses tool. http://software.broadinstitute.org/gsea/msigdb/index.jsp). **a and b** show the pathway enrichment output generated using the Hallmark and KEGG curated gene sets respectively.



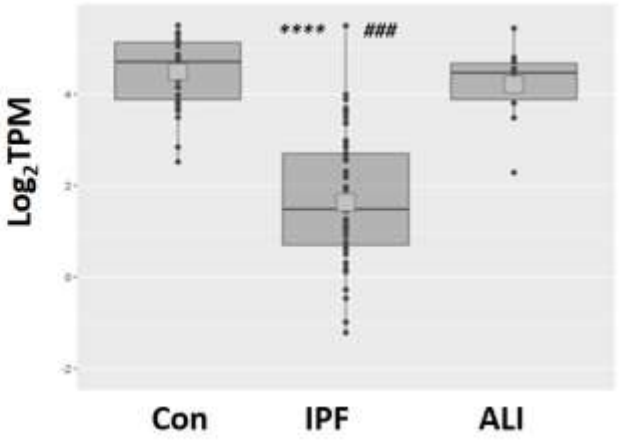
<u>Supplementary Figure S2. Cholesterol homeostasis pathway in advanced IPF lung.</u> a. List of top genes within the cholesterol homeostasis pathway modulated in IPF vs healthy control as analyzed by Hallmark curated gene sets within the msigdb tool. **b.** Overlap of cholesterol homeostasis pathway genes modulated in our advanced IPF cohort (left) with genes modulated by statin treatment in lung tissues (right, Lane et al., 2015). **c** gene expression of PCSK9, a cholesterol homeostasis protein, in IPF lung, as compared to healthy and ALI samples (adjusted P-value ****** 5.06 E16 IPF vs Con, *###* 4.4 E9 IPF vs ALI).

a)

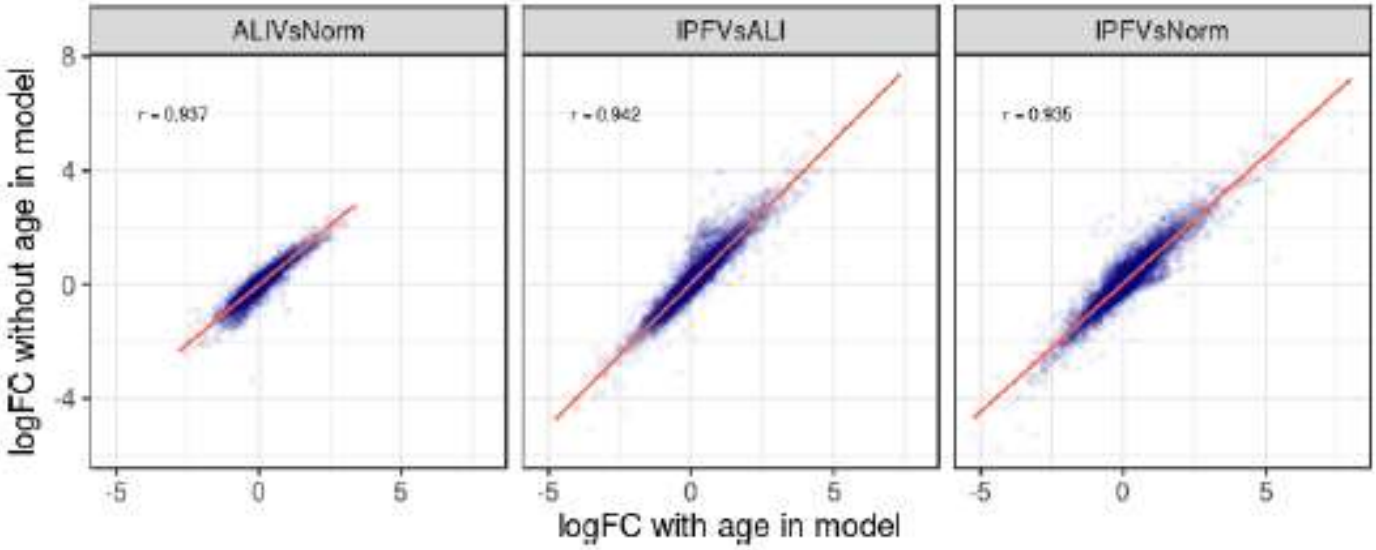
Gene	Fold Change	adj p Value
CBS	-2.225481498	2.35E-10
TMEM97	-2.030222108	4.51E-20
SCD	-2.000419122	1.33E-13
FASN	-1.975888669	4.24E-16
HMGCS1	-1.768781662	1.89E-17
TRIB3	-1.564892709	2.37E-09
SQLE	-1.541730289	1.39E-12
CYP51A1	-1.467395622	6.29E-16
LPL	-1.407602127	4.81E-08
ACAT2	-1.396271868	1.43E-13
MAL2	-1.352158625	4.31E-14
DHCR7	-1.33270704	9.24E-11
HMGCR	-1.240369563	2.59E-14
EBP	-1.207899315	2.28E-14
LSS	-1.188249428	8.82E-17
PCYT2	-1.171103614	4.00E-19
IDI1	-1.121497616	1.38E-13
SC5D	-1.117679903	1.27E-13
STARD4	-1.11535601	2.00E-15
ACSS2	-1.083691985	8.68E-19
ALCAM	-1.036146121	2.36E-16
MVD	-1.030535482	2.35E-12
HSD17B7	-0.961687444	6.54E-14
FDPS	-0.943309546	8.74E-15
TM7SF2	-0.942010127	1.63E-13
FDFT1	-0.903185575	1.26E-14
SREBF2	-0.783668211	1.59E-12
NSDHL	-0.770727741	8.69E-14
ERRFI1	-0.751211381	3.42E-06
FABP5	-0.706864901	7.60E-07
MVK	-0.668561675	9.86E-11
GSTM2	0.599113333	2.42E-06
ABCA2	0.663111148	1.52E-12
PLAUR	0.730837952	0.000136493
TP53INP1	0.768159204	4.10E-06
FAM129A	0.768734364	1.90E-12
GLDC	0.916244332	0.003609529
GPX8	1.346343622	7.27E-14
ATF3	1.961521494	2.40E-09
AVPR1A	2.47637665	1.29E-09

b)

c)



Supplementary Figure S3: High Correlation of Gene Expression Fold Changes With and Without Age in the Model



Supplementary Figure S4: Age effect on Pathway Enrichment in IPF vs Control and ALI Vs Control Contrasts:
Note that the inclusion of age in the statistical model does not alter the pathway regulation results presented in the paper. Cholesterol biosynthesis, T-cell cosignaling, Cell Adhesion, EMT and ECM remodeling are still enriched in IPF cohort while cell cycle pathways are primarily enriched in the ALI cohort