Supplemental data

Material and methods:

Metabolomics: Metabolomic analysis was performed as previously described [7]. Briefly, samples were performed using the automated MicroLab STAR® system (Hamilton Co, Reno, NV, USA). Samples were placed on a TurboVap® (Zymark) to remove the organic solvent and each sample was frozen and dried under vacuum conditions and were prepared for either UPLC/MS/MS or GC/MS. Raw data was extracted, peak-identified, and QC was processed using Metabolon's hardware and software. Compounds were identified by comparison to library entries of purified standards or recurrent unknown entities. More than 2400 commercially available purified standard compounds have been acquired and registered into LIMS for distribution to both the LC and GC platforms for determination of their analytical characteristics (http://www.metabolon.com/about-us.aspx)

Summary and Significantly Altered Biochemicals: The present dataset comprises a total 108 detectable compounds of known identity (named biochemicals). Following log transformation and imputation with minimum observed values for each compound, Welch's two-sample t-test was used to identify biochemicals that differed significantly between experimental groups comparing lung sample from both control and IPF groups. A summary of the numbers of biochemicals that achieved statistical significance ($p \leq 0.05$), as well as those approaching significance ($0.05 < p < 0.10$). Pathways were assigned for each metabolite in order to examine the impact of an increased or decreased metabolite on the overall pathway.

An estimate of the false discovery rate (q-value) is calculated to take into account the multiple comparisons that normally occur in metabolomic-based studies. For example, when analyzing 200 compounds, we would expect to see about 10 compounds meeting the $p \leq 0.05$ cut-off by random chance. The q-value describes the false discovery rate; a low q-value ($q < 0.10$) is an indication of high confidence in a result. While a higher q-value indicates diminished confidence, it does not necessarily rule out the significance of a result. Other lines of evidence may be taken into consideration when determining whether a result merits further scrutiny. Such evidence may include a) significance in another dimension of the study, b) inclusion in a common pathway with a highly significant compound, or c) residing in a similar functional biochemical family with other significant compounds.

Transcriptomic analysis: Global profiles were determined in human lung tissue and compared across normal and idiopathic pulmonary arterial hypertension patients 1. Biotinylated RNA was prepared according to the standard Affymetrix protocol. cRNA was hybridized on GeneChip Genome Array and geneChips were scanned and the data were analyzed with Partek Genomics Suite 6.6. The value definition was set up using Partek Genomics Suite 6.6. Statistical analysis of log-transformed data was performed using "R" (http://cran.r-project.org/), which is a freely available, open-source software package. Welch's t-tests were performed to compare data between control and IPF groups. Significance changes based on the most stringent criteria (intersection of $p \leq 0.05$ and ≥ 2 fold change), the up-regulated/down-regulated genes encoding to metabolic related enzymes were observed in both groups. The database has been submitted to NCBI/GEO and has been approved and assigned a GEO accession number, GSE53408.

Supplement Figure 1 a) The pentose phosphate pathway is shown. Data for metabolic intermediates in the normal lung are shown in green boxes and data for IPF are represented in purple boxes. This pathway uses the glycolytic intermediate glucose 6-phosphate to produce ribose for nucleotide synthesis. It can

produce glyceraldehyde 3-phosphate and fructose 6-phosphate for glycolysis. Red arrows beside metabolites indicate upward trends in metabolite levels in IPF compared to normal, but not significant increases. b) Ribulose 5-phosphate, xylulose 5-phosphate, and ribose levels were found increased in IPF lungs compared to control. This may hint at shuttling of glycolytic metabolites toward the pentose phosphate pathway for nucleotide synthesis. Y axis label of metabolic changes as counts X 10^6 and the Y axis label for gene expression encoding enzymes as fold changes of relative RNA levels of enzymes.

Supplemental Figure 2. a), Fatty acid –oxidation for dicarboxylic acid production. There was a significant accumulation of long- and medium-chain fatty acids, including caproate, caprylate, myristate, and palmitoleate in IPF lungs compared to control. Succinate looks to be increased, but not significantly. We may need to do statistical analysis again. Increased fatty acid concentrations with no concomitant increase in dicarboxylic acids or succinate indicate that omega oxidation is likely unchanged in IPF. Y axis label of metabolic changes as counts X 10^6.

Supplemental Figure 3. Mitochondrial Transport. In all graphs, gene encoding metabolic enzymes for normal lung are shown in blue open boxes and data for IPF lung are represented in green open boxes. Genes encoding carnitine shuttle were found significantly decreased, including decreased expression of Acyl-CoA Thioesterase 1, 2, 8, 13 (ACOT 1, 2, 8, 13). We also found increased expression of ACAD11 but decreased Acyl-CoA Dehydrogenase, C-2 To C-3 Short Chain (ACADS) in IPF lungs compared to normal (ACADS, p = 3.57e-10; ACAD11, p = 1.68e-14; ACOT1, p = 1.16e-7; ACOT2, p = 2.19e-4; ACOT8, p = 1.21e-10; ACOT13, p = 4.27e-16; CS, p = 5.6e-15). Y axis label of metabolic changes as counts X 10^6 and the Y axis label for gene expression encoding enzymes as fold changes of relative RNA levels of enzymes.

Supplemental Figure 4. Glutamine and Aspartate metabolism. In all graphs, gene encoding metabolic enzymes for normal lung are shown in blue open boxes and data for IPF lung are represented in green open boxes while metabolic data for control lung are shown in green boxes. Significantly increased glutamate, glutamine and glutathione (GSH) with decreased levels of its oxidized counterpart GSSG and aspartate levels were found in IPF compared to normal. We also found decreased gene expression levels of glutamate dehydrogenase 1 (GLUD1) in IPF (p = 1.3e-8). Increased glutamine and glutamate, with downregulated GLUD1 gene, may reflect shuttling of metabolites away from the TCA cycle and towards glutathione production and increased GSH may reflect the cell's attempt to increase responses to oxidative stress

Supplemental Figure 5. The panels display morphologic changes in the IPF lung with hematoxylin-eosin staining. The pulmonary images of four images from IPF lung showing a disorganized lung tissue with full fibrotic tissues within perivascular and airway tissues. (n=4, magnification 5x).