## **Supplementary Information**

Metformin induces lipogenic differentiation in myofibroblasts to reverse lung fibrosis

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**Supplementary Figure 1. Dose-finding study for treating human IPF lung fibroblasts with metformin. (A-C)** qPCR analysis of *PLIN2, PPARg* and *COL1A1* in IPF fibroblasts treated with 1 mM, 5 mM or 10 mM metformin or vehicle. (**D**) Western blot showing the expression of the apoptotic marker cleaved PARP1 in fibroblasts treated with vehicle or 5 mM metformin. Quantification of the immunoblot is shown in the right panel. Each data point corresponds to one patient and error bars indicate s.e.m. (A-C) n=6-7 per group, (D): n=3 per group. Kruskal-Wallis test was used in (A-C) and Mann-Whitney test was used in (D). \* P<0.05, \*\*P<0.01, \*\*\*\*P<0.0001. ns: Not significant.



Supplementary Figure 2. Metformin induces lipogenic differentiation in IPF fibroblasts starting at 72 h after treatment. (A) Schematic representation of the experimental setup. (B-D) qPCR analysis of *PLIN2, PPARg* and *COL1A1* in IPF fibroblasts treated with 5 mM metformin for 48 h. A Similar analysis is shown after 72 (E-G) and 96 h (H-J). (K-P) Staining of metformin- and vehicle-treated cells with LipidTOX (red) and DAPI (blue) at 48, 72 and 96 h. (Q-S) Quantification of lipid-droplet accumulation is shown in (K-P). Scale bars: (K-P) 25 µm. Each data point corresponds to one patient and error bars indicate s.e.m. (B-J) n=4-5 per group. (Q-S) n=4 per group. Mann-Whitney test was used in (B-J, Q-S). \* P<0.05.

- Signaling pathways regulating pluripotency of stem cells RIG-I-like receptor signaling pathway Dilated cardiomyopathy
- ۲
- Histidine metabolism
- Phenylalanine metabolism
- Pancreatic secretion
   Glutathione metabolism
  - Axon guidance
  - Synaptic vesicle cycle
  - Légionellosis
  - Leukocyte transendothelial migration
     Fatty acid biosynthesis
     Fatty acid degradation

  - Adherens junction
  - Insulin signaling pathway

  - ErbB signaling pathway Selenocompound metabolism
  - Rap1 signaling pathway
     Purine metabolism

  - Nucleotide excision repair ۲
  - Phototransduction Protein digestion and absorption
  - Platelet activation
  - Basal transcription factors

  - Hedgehog signaling pathway
    Bacterial invasion of epithelial cells
    Jak-STAT signaling pathway
    Linoleic acid metabolism

  - Glycine, serine and threonine metabolism

  - Focal adhesion
     Wnt signaling pathway
     Basal cell carcinoma
  - Fatty acid elongation
  - Starch and sucrose metabolism
  - Antigen processing and presentation
    Glycerolipid metabolism
    Chemical carcinogenesis

  - Epithelial cell signaling in Helicobacter pylori infection
     Metabolism of xenobiotics by cytochrome P450
     NF-kappa B signaling pathway
     Collecting duct acid secretion
     Morphine addiction

  - Dorso-ventral axis formation
  - Biosynthesis of unsaturated fatty acids
  - Non-homologous end-joining
     TGF-beta signaling pathway
    - Alcoholism
    - Glycolysis / Gluconeogenesis
       Proteoglycans in cancer
       TNF signaling pathway
       Rheumatoid arthritis

    - - NOD-like receptor signaling pathway
      - Ubiquitin mediated proteolysis

      - RNA transport
         RNA transport
         Thyroid hormone signaling pathway
         Pathogenic Escherichia coli infection
         N-Glycan biosynthesis

      - Retinol metabolism
- Fatty acid metabolism
   Terpenoid backbone biosynthesis
   Hippo signaling pathway
   PPAR signaling pathway
   Drug metabolism cytochrome P450
  - Peroxisome



- Transcriptional misregulation in cancer
   MicroRNAs in cancer
   Ribosome biogenesis in eukaryotes

Ribosome

Systemic lupus erythematosus







 $-\log_{10}(p)$ 

0

Supplementary Figure 3. KEGG significance plot showing signaling pathway alterations in metformin- and vehicle-treated human IPF lung fibroblasts. The top 75 candidate pathways are shown. Selected pathways are marked by the arrows. Vehicle-treated group: n=4, metformin-treated group: n=3.



**Supplementary Figure 4.** *BMP2* knockdown inhibits metformin-induced lipogenic differentiation in IPF lung fibroblasts. (A) Schematic representation of the experimental setup. (B-E) qPCR analysis of *BMP2*, *PLIN2*, *PPARg* and *COL1A1* in human IPF lung fibroblasts treated with metformin in the presence of scramble or *BMP2* siRNA. Each data point corresponds to one patient and error bars indicate s.e.m. n=4 per group. Mann-Whitney test was used in (B) and Kruskal-Wallis test was used in (C-E). \* P<0.05. Met: Metformin, ns: Not significant, Veh: Vehicle.



Supplementary Figure 5. Inhibition of ERK phosphorylation does not mediate the antifibrotic effect of metformin in human IPF lung fibroblasts. (A) Kinase activity assay as determined by PamStation analysis. (B) Western blot showing decreased p-ERK levels in response to metformin treatment. Quantification of p-ERK levels is shown in the lower panel. (C) Schematic representation of the experimental setup. (D-F) qPCR analysis of *PLIN2, PPARg* and *COL1A1* in IPF fibroblasts treated with selumetinib or vehicle for 72 h. Each data point corresponds to one patient and error bars indicate s.e.m. (B) n=6 per group. (D-F) Vehicle-treated group: n=5, Selumetinib-treated group: n=6. Mann-Whitney test was used. \* P<0.05. ns: Not significant.



Supplementary Figure 6. Nintedanib and pirfenidone do not induce lipogenic differentiation in IPF lung fibroblasts. (A) Schematic representation of the experimental setup. (B-D) qPCR analysis of *PLIN2, PPARg* and *COL1A1* in human IPF lung fibroblasts treated with nintedanib. A similar analysis for pirfenidone-treated cells is shown in (E-G). (H-J) Staining of nintedanib-, pirfenidone- and vehicle-treated cells with LipidTOX (red) and DAPI (blue). (K) Schematic representation of the experimental setup using precision-cut lung slices (PCLS). (L-N) 3D-reconstruction of z-stacks of nintedanib-, pirfenidone- and vehicle-treated PCLS stained for COL1A1 (green) and lipid droplets (red). (O) Quantification of LipidTOX<sup>+</sup> cells as determined by manual counting across z-stacks. Scale bars: (H-J) 50 µm. (B-G) Each data point within a given group corresponds to one patient. (B-D) Vehicle-treated group: n=5, nintedanib-treated group: n=6. (E-G) n=4 per group. (O) Each data point within a given group corresponds to one z-stack and error bars indicate s.e.m. Vehicle-treated group: n=7, nintedanib-treated group: n=6, pirfenidone-treated group: n=8. Mann-Whitney test was used in (B-G) and Kruskal-Wallis test was used in (O). \* P<0.05.