Figure S1

(a) GSE24206 / GPL570 / 200998.at

(b) GSE13330 / GPL570 / 200998.at

(c) Gene expression for UCP2 (ENSG000000175567.8)
Figure S1. UCP2 is highly expressed in IPF lungs and senescent fibroblasts.

(a) A publicly available GEO dataset, GSE24206 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE24206] was queried for UCP2 gene expression in whole lung tissue derived from non-IPF patients and those with early and advanced IPF using the GEO2R tool, graphical output shown. (b) A publicly available GEO dataset, GSE13330 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE13330] was queried for UCP2 gene expression in human foreskin fibroblasts that were either non-senescent or subjected to replicative senescence or stress-induced senescence (via exposure to bleomycin) using the GEO2R tool, graphical output shown. (c) UCP2 gene expression in various normal tissues in humans was obtained from the Genotype-Tissue Expression (GTEx) project online portal [https://www.gtexportal.org/home/gene/UCP2]. The graphical output is in log scale sorted in ascending order of median expression in tissues. (d-h) The Fibroexplorer dataset (an online resource tool derived from fibroblast atlases by integration of single-cell transcriptomic data from approximately 230,000 fibroblasts across 17 tissues, 50 datasets, 11 disease states and 2 species) for perturbed state (human), available at [https://www.fibroxplorer.com/perturbed-state-human], was queried for UCP2 gene expression; output shown. (d) shows the clusters of fibroblasts characterized in the dataset (X axis) in various conditions (Y axis) (ulcerative colitis; PDAC = pancreatic ductal adenocarcinoma; NSCLC = non-small cell lung cancer; IPF = idiopathic pulmonary fibrosis; COVID = covid-19 pneumonia; the latter three fibroblast sets isolated from lungs). The sizes of the black boxes show relative expression of UCP2 in the cluster/condition. (e) shows relative expression of UCP2 in the various clusters in violin plots. (f) shows the Uniform Manifold Approximation and Projection (UMAP) plot of the various clusters in the dataset, with each cluster showing a different color. (g) shows the relative expression of UCP2 among the various clusters in the UMAP plot, with predominant expression in the NPNT cluster. (h) shows the relative expression of top genes in the clusters of cells limited by the selection (blue box) in (g).
Figure S2. Efficacy of UCP2 siRNA.

An siRNA sequence targeting human UCP2 was designed, and its efficacy tested on non-IPF and IPF fibroblasts (derived from one lung explant each) (a). The fibroblasts were treated with the UCP2 siRNA or non-targeting (NT) siRNA (100 nM each) for 72 hours; steady-state mRNA levels assessed by qPCR. Graph represents mean ± SEM (n=3 each group); ***p<0.001. (b) IPF fibroblasts were treated with UCP2 or NT siRNA for 72 hours; western blotting was performed to assess steady-state expression of UCP2; representative blots shown, with their densitometric analysis in (c); error bars represent mean ± SEM (n=3); **p<0.01. Please note that the UCP2 band appears at ~95 kDa which represents 3x molecular weight of the UCP2 monomer. (d, e) A mouse/rat siRNA sequence that is orthologous to the human UCP2 siRNA sequence was generated. This sequence works for both mouse (Mus musculus) and rat (Rattus norvegicus) and differs from the human sequence in only 2 (out of 19) bases (see Key Resources Table). Mouse lung fibroblasts (d) and rat lung epithelial (RLE) cells (e) were treated with this siRNA (or non-targeting siRNA) for 72 hours; steady-state mRNA levels assessed by qPCR. Graphs represent mean ± SEM (n=3 each group); **p<0.01, ***p<0.001. NT = non-targeting.
Figure S3. Heatmap of metabolites in IPF fibroblasts treated with UCP2 siRNA.

IPF fibroblasts were subjected to siRNA-mediated knockdown of UCP2 for 72 hours. Lysates (experimental triplicate) were subjected to metabolomics analyses (same experiment described in Figure 3d, e). A heatmap of the top 25 metabolites that are significantly different between the two groups is shown; metabolites with lower concentrations are in blue and those with higher concentrations are in red. NT = non-targeting.
Figure S4

(a) O/R

(b) O/R

(c) O/R

(d) O/R

(e) O/R

(f) O/R

(g) O/R
Figure S4 continued...

Figure S4. Mitochondrial Stress Test in IPF fibroblasts subjected to UCP2 silencing.

(a, b) IPF fibroblasts were subjected to siRNA-mediated silencing of UCP2 for a total of 72 hours. The fibroblasts were seeded 15,000 cells per well prior to measuring oxygen consumption rate (OCR) in an XFe96 analyzer (Seahorse Bioscience); graphical output depicted in (a); all individual components of OCR represented in (b). Error bars represent mean ± SEM (n=8); *p<0.05, **p<0.01. (c) IPF fibroblasts were subjected to siRNA-mediated silencing of UCP2 for a total of 72 hours, seeded at 15,000 cells per well, incubated with a substrate-restricted medium and treated with etomoxir 4 μM or vehicle at the indicated time point. OCR was measured; graphical output depicted. (d) IPF fibroblasts were subjected to siRNA-mediated silencing of UCP2 for a total of 72 hours, seeded at 15,000 cells per well, incubated with a substrate-restricted medium and then treated with bovine serum albumin (BSA) or BSA-Palmitate conjugate as per kit manufacturer’s instructions, prior to measuring OCR; graphical output depicted. (e) IPF fibroblasts were subjected to siRNA-mediated silencing of UCP2, CPT1α or both for a total of 72 hours. The fibroblasts were seeded 15,000 cells per well and incubated in a substrate restricted medium prior to measuring OCR; graphical output depicted. (f, g) IPF fibroblasts with similar experimental conditions as in (A-B) were assessed for extracellular acidification rate (ECAR) in an XFe96 analyzer; graphical output depicted in (f); all individual components of ECAR represented in (g). Error bars represent mean ± SEM (n=8); *p<0.05, ***p<0.001. In all the studies above, blue arrows indicate the time points when etomoxir (Eto), oligomycin (O), Carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP) (F), antimycin A/Rotenone (A/R) and 2-deoxy glucose (2-DG) were added to the cells as part of the Seahorse Mito Stress Test. (h) IPF fibroblasts were subjected to siRNA-mediated silencing of UCP2 for 72 hours. Mitochondrial DNA (mtDNA) content was assessed; graph shows relative expression compared to average of mtDNA content in fibroblasts treated with NT siRNA; mean ± SEM (n=3); difference statistically not significant. (i) IPF fibroblasts were subjected to siRNA-mediated silencing of UCP2 for 72 hours. Western blotting was performed to assess steady-state levels of proteins involved in mitochondrial oxidative phosphorylation – complex V (ATP synthase α; ATPSα), complex III (ubiquinol-cytochrome c reductase core protein 2; UQCRC2) and complex II (succinate dehydrogenase B; SDHB), using the Total OXPHOS Human WB Antibody Cocktail from Abcam. NT = non-targeting.
Figure S5. Effects of targeting UCP2 on IPF fibroblast senescence and myofibroblast differentiation.

(a) SA-β-gal staining was performed on replicatively senescent (high population doubling length – PDL >40) IMR-90 fibroblasts subjected to siRNA-mediated silencing of UCP2 for 72 hours; representative light microscopy images (10X) shown. (b) Fibroblasts derived from two different IPF lung explants, one with low and the other with high expression of myofibroblast markers α-smooth muscle actin (α-SMA) and collagen 1a1 (COL1a1), were treated with UCP2- (or non-targeting, NT-) siRNA for 72 hours. Western blotting was performed to assess steady-state levels of myofibroblast markers (α-SMA and COL1a1) and proliferation marker pRb. (c, d) IPF fibroblasts were treated with UCP2 inhibitor genipin 250 μM for 48 hours. Gene expression of myofibroblast markers α-smooth muscle actin (α-SMA) (c) and collagen 1a1 (COL1a1) (d) were assessed by qPCR. Graphs represent mean ± SEM (n=4 each group); ***p<0.001, ****p<0.0001.
Figure S6

(a) GSE6591 / GPL1251 / 1459741_X_at

Young - 2m   Old - 18m   Older - 26m

Expression value

(b) Lung UCP2

(c) Lung COL1a1

(d) Static Lung Compliance

(e) Bleo + NT siRNA

(f) Bleo + UCP2 siRNA

(g) MPO/T1-α/DAPI

(h) Fibroblast α-SMA

(i) Fibroblast COL1a1

(j) Mouse Lung Fibroblasts

Bleo + NT siRNA Bleo + UCP2 siRNA

NT                   UCP2
β-Actin
α-SMA
COL1a1
siRNA

MPO/T1-α/DAPI

H&E

β-Actin

α-SMA

COL1a1

siRNA

NT                   UCP2

MPO/T1-α/DAPI

β-Actin

α-SMA

COL1a1

siRNA
Figure S6. UCP2 expression lungs and lung fibroblasts of aged mice and therapeutic targeting of UCP2 in aged mice subjected to bleomycin injury.

(a) A publicly available GEO dataset, GSE6591 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE6591), was queried for UCP2 gene expression in the lungs among various age groups in C57BL/6J mice using the GEO2R tool; graphical output shown. (b-g) In the experiment described in Figure 6b, whole lung lysates (from the aged mice subjected to bleomycin injury and then treated with UCP2- or non-targeting siRNA) were assessed for gene expression of UCP2 (b) and collagen 1a1 (c) by qPCR. Graphs represent mean ± SEM (n=3 each group); *p<0.05. (d) Lung function was assessed using the flexiVent™ apparatus; static compliance measured; graph represents mean ± SEM (n=4-5 each group); ***p<0.001. (e, f) Representative images show the areas of fibrotic remodeling and fluorescence patterns of myeloperoxidase (MPO, red), type 1 epithelial cells (T1-α, green) and nuclei (DAPI-blue) in indicated groups of mice. Scale bars = 50 μm. (g) Fibroblasts were isolated from the lungs and western blotting was performed to assess the steady state levels of myofibroblast markers α-smooth muscle actin (α-SMA) and collagen 1a1 (COL1a1); densitometric analyses shown in (h) and (i) respectively; graphs represent mean ± SEM (n=3); *p<0.05, **p<0.01. (j) In a separate experiment, young (2 months) and aged (18 months old) C57BL/6 mice were subjected to lung injury by instillation of oropharyngeal bleomycin (1.5 U/kg) (or no injury by instillation of PBS control). Lungs were harvested starting at 3 weeks up to 2 months after bleomycin injury; fibroblasts were isolated from the lungs and assessed for gene expression of UCP2 and collagen 1a1 (COL1a1). The graph represents correlation between mRNA expression of UCP2 and COL1a1.
High constitutive levels of uncoupling protein-2 (UCP2) are observed with aging and in the age-related fibrotic disease, idiopathic pulmonary fibrosis (IPF). UCP2 functions as an exporter of fatty acid (FA) anions, which may account for the observed decrease in mitochondrial membrane potential of IPF myofibroblasts. Decreased coupling efficiency and fatty acid oxidation (FAO) lower ATP synthetic capacity in association with increased reactive oxygen species (ROS) production. These alterations promote induction of senescence and apoptosis resistance of myofibroblasts, hallmarks of recalcitrant and progressive fibrosis as seen in IPF.
| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Antibodies and Stains |        |            |
| UCP2 (immunohistochemistry) | Proteintech | 11081 |
| UCP2 (western blotting) | Santa Cruz | sc-6525 |
| Cyclin D1 | Santa Cruz | sc-8396 |
| pRb | Cell Signaling | 9308s |
| α-SMA | ARP | 03-61001 |
| COL1a1 | Abcam | ab138492 |
| GAPDH | Abcam | ab9445 |
| Cleaved PARP | Cell Signaling | 9541 |
| p16 | Cell Signaling | 80772S |
| Total OXPHOS Human WB Antibody Cocktail | Abcam | ab110411 |
| β-Actin | Sigma | A5316 |
| JC-1 Dye | Invitrogen | T3168 |
| LipidTOX | Invitrogen | H34351 |
| MitoSOX™ | Invitrogen | M36008 |
| MitoTracker™ Green | Invitrogen | M7514 |
| DAPI | Thermo Scientific | 9541s |
| Ki-67 | BD Biosciences | 550609 |
| TUNEL | Roche | 12156792910 |
| MPO | Abcam | ab208670 |
| T1-α (Mouse podoplanin) | R&D Systems | AF3244 |
| Chemicals, peptides, and recombinant proteins |        |            |
| DMEM/F-12 | Gibco | 11320033 |
| Fetal Bovine Serum | Gibco | 10437028 |
| Pen Strep | Gibco | 15140 |
| Trypsin-EDTA (0.25%) | Gibco | 25200 |
| DPBS pH 7.4 | Gibco | 14190 |
| OptiMEM medium | Gibco | 11058021 |
| Lipofectamine 2000 | Thermofisher | 11668019 |
| Dimethyl Sulphoxide (DMSO) TC grade | Sigma Aldrich | D2650-100ML |
| Nitrocellulose blotting membrane 0.45 mm | GE Healthcare Amersham | 10600002 |
| HEPES | Gibco | 15630080 |
| TRITON X-100 | Sigma | T9284 |
| The Dako Envision Dual Link System | Agilent | K406311-2 |
| DAB/H2O2 kit | Vector Laboratories | SK-4100 |
| Tween-20 | Sigma Aldrich | P1379-4L |
| Item                                               | Supplier               | Code/Number         |
|----------------------------------------------------|------------------------|---------------------|
| Collagenase type I                                 | Advanced Biometrix     | 5005-100ml          |
| Isoflurane                                         | Piramal                | NDC 66794-017-25    |
| SYBR Green power up                               | Applied Biosystems     | A25742              |
| Bleomycin                                          | Meitheal               | NDC 71288-106-10    |
| 5X siRNA Buffer                                   | Horizon Discovery      | B-002000-UB-100     |
| Hematoxylin                                        | Vector Laboratories    | H-3401-500          |
| Masson's trichrome staining                        | Sigma                  | HT15-1KT            |
| TRIS BASE                                          | Fisher                 | BP1521              |
| EDTA                                               | BP118500               | Fisher              |
| Halt™ Protease and Phosphatase Inhibitor           | Thermo Scientific      | 78442               |
| RIPA                                               | Thermo Scientific      | 89901               |
| Diethylenetriaminepentaacetic acid (DTPA)          | Sigma Aldrich          | D6518-5G            |
| Sodium chloride (NaCl)                             | Fisher Scientific      | BP358-212           |
| Potassium phosphate dibasic (HK₂PO₄)               | Fisher Scientific      | BP363-500           |
| Potassium phosphate monobasic (KH₂PO₄)             | Sigma Aldrich          | P-5379              |
| Krebs-Henseleit buffer (KHB)                       | (Alfa Aesar, Hill)     | J67820              |
| mito-TEMPO-H                                       | Enzo Life Sciences     | ALX-430-171-M005    |
| Genipin                                            | Sigma                  | G4796               |
| XF calibrant solution                              | Agilent Technologies   | 100840-000          |
| XF DMEM medium                                     | Agilent Technologies   | 103575-100          |
| 100 mM pyruvate solution                           | Agilent Technologies   | 103578-100          |
| 200 mM glutamine                                   | Agilent Technologies   | 103579-100          |
| D-Glucose                                          | Agilent Technologies   | 103577-100          |
| Oligomycin                                         | Sigma                  | O4876               |
| FCCP                                               | Sigma                  | C2920               |
| Rotenone                                           | Sigma                  | R8875               |
| Antimycin A                                        | Sigma                  | A8674               |
| 2-deoxy-D-glucose                                  | Fisher                 | AC111980050         |
| Etomoxir                                           | Cayman Chemicals       | 11969               |

**Critical commercial assays**

| Item                                               | Supplier               | Code/Number         |
|----------------------------------------------------|------------------------|---------------------|
| Pierce BCA protein assay kit                        | Thermo Scientific      | 23225               |
| RNeasy Mini Kit                                    | QIAGEN                 | 74104               |
| Product Name                        | Manufacturer | Catalog Number |
|------------------------------------|--------------|----------------|
| iScript reverse transcription kit   | Bio-Rad      | 1708841        |
| ATP Assay Kit                      | Abcam        | ab83355        |
| Deproteinizing Sample Preparation Kit | Abcam    | ab204708       |
| Fluorometric hydroxyproline assay  | QuickZyme Biosciences | QZBHYPRO1 |
| Seahorse XFe96 Cell Culture Microplate | Agilent Technologies | 101085-004    |
| Seahorse XFe96 Extracellular FluxPak mini | Agilent Technologies | 102601-100 |
| Seahorse XF Palmitate-BSA FAO Substrate | Agilent Technologies | 102720-100    |

**Experimental models: Cell lines**

| Model       | Source     | Catalog Number |
|-------------|------------|----------------|
| IMR-90      | ATCC       | ATCC® CCL-186™ |

**Experimental models: Organisms/Strains**

| Organism   | Source   | Catalog Number |
|------------|----------|----------------|
| Mouse: C57BL/6J | Jackson Laboratories      | Cat# 000664 |

**Oligonucleotides – primers**

| Primer Name   | Sequence                | Source            | Catalog Number |
|---------------|-------------------------|-------------------|----------------|
| UCP2 (Forward): | ACA AGA CCA TTG CCC GAG AG | This manuscript | N/A            |
| UCP2 (Reverse): | AGG AGG GCA TGA ACC CTT TG | This manuscript | N/A            |
| α-SMA (Forward): | TCC TCA TCC CTT GAG AA | This manuscript | N/A            |
| α-SMA (Reverse): | ATG AAG GAT GGC TGG AAC AG | This manuscript | N/A            |
| COL1A1 (Forward): | ACG AAG ACA TCC CAC CAA TCA CCT | This manuscript | N/A            |
| COL1A1 (Reverse): | AGA TCA CGT CAT CGC ACA ACA CCT | This manuscript | N/A            |
| GAPDH (Forward): | ATC ATC CCT GCC TCT ACT GG | This manuscript | N/A            |
| GAPDH (Reverse): | CTG CTT CAC CAC CTT CTT GA | This manuscript | N/A            |
| β-actin (Forward): | TGC TAT CCA GGC TGT GCT AT | This manuscript | N/A            |
| β-actin (Reverse): | AGT CCA TCA CGA TGC CAG T | This manuscript | N/A            |
| Mouse UCP2 (Forward): | AAG TGT TTC GTC TCC CAG CC | This manuscript | N/A            |
| Mouse UCP2 (Reverse): | GGG ACC TTC AAT CGG CAA GA | This manuscript | N/A            |
| Oligonucleotides - siRNAs                  |                      |                      |
|------------------------------------------|----------------------|----------------------|
| Human UCP2 siRNA (sense):                | This manuscript      | N/A                  |
| AAU GCC UAC AAG ACC AUU G                 |                      |                      |
| Mouse/Rat UCP2 siRNA (sense):            | This manuscript      | N/A                  |
| GAA GCC UAC AAG ACC AUU G                 |                      |                      |
| Human CPT1α siRNA (sense):               | This manuscript      | N/A                  |
| GGG AGA GAA CCU CAU CAA AUU              |                      |                      |
| Non-targeting siRNA (sense):             | This manuscript      | N/A                  |
| UAA GGC UAU GAA GAG AUA C                 |                      |                      |

| Software and algorithms                  |                      |                      |
|------------------------------------------|----------------------|----------------------|
| Prism 9.4                                 | Graphpad software    | https://graphpad.com  |
| MetaboAnalyst 5.0                        | Open source software | http://metaboanalyst.ca |
| FlowJo                                   | FlowJo software      | https://www.flowjo.com/ |
| Fiji                                     | Open source software | https://imagej.net/Fiji |
| fibroXplorer                              | Open source software | https://www.fibroxplorer.com/perturbed-state-human |

| Other                                    |                      |                      |
|------------------------------------------|----------------------|----------------------|
| Keyence BZ-X700 microscope               | Keyence              | N/A                  |
| LSR-II flow cytometer                    | Becton Dickinson     | N/A                  |
| XFe96 extracellular flux analyzer        | Seahorse Biosciences | N/A                  |