Supplemental Figure 1. Genetic knockout of Drp1 decreases oxygen consumption rates (OCR) and tumor growth.

(A) Mitochondrial morphology in control sgGFP cells and sgDrp1 cells with a quantified morphology percentage, 60X magnification, scale bar: 10 μm, cells used in quantification n = 100-200. ****P = 0.0001 by unpaired t test. (B) Mito stress assay of control cells and Drp1 knockout cells. Data were normalized to protein concentration. (C) Quantified bar chart of (B). ****P < 0.0001 by unpaired t test. (D) In vitro cell proliferation of control and Drp1 knockout cells. Statistical analysis by 2-way ANOVA. (E) Knockout of Drp1 induces cell cycle arrest at G1. ***P < 0.001 by 2-way ANOVA. (F) Kaplan-Meier survival curves of C57BL/6J mice bearing orthotopic pancreatic cancer tumor, n = 5 per cohort. Statistical analysis by Gehan-Breslow-Wilcoxon test. (G) Immunoblot of orthotopic tumors confirming Drp1 knockout. Data are presented as mean ± SEM.
Supplemental Figure 2. Anti-tumorigenic effect of Mdivi-1
(A) KPC cells treated with Mdivi-1 exhibit elevated levels sub G_0/G_1 phase, indicating more apoptosis. ***P < 0.001, ****P < 0.0001 by 2-way ANOVA. (B) TUNEL assay characterizing apoptotic activity of Mdivi-1. Scale bar: 20 µm. (C) Quantification of apoptotic cells after Mdivi-1 treatment. (*Vehicle control group is shared with Supplemental Figure 5D). Statistical analysis by unpaired t test. Data are presented as mean ± SEM.
Supplemental Figure 3. Doxycycline induces gene expression over a wide range of concentrations.

(A) Mitochondrial morphology of KPC cells with/without Mfn2 expression, scale bar: 10 μm, morphology percentages quantified. Cells used for quantification n = 100-200. ***P = 0.0002 for tubular, *P = 0.04 for intermediate, **P = 0.001 fragmented by unpaired t test. (B) In vitro cell proliferation of control (Mfn2 OFF) and Mfn2 overexpression cells (Mfn2 ON). Statistical analysis by 2-way ANOVA. (C) Overexpression of Mfn2 induces cell cycle arrest at G1. **P < 0.01 ***P < 0.001 by 2-way ANOVA. (D) In vivo KPC tumor growth by feeding mice with different concentration of doxycycline water. n = 5 per cohort. (E) Mice weight during tumor growing. Weighed twice a week. (F) Mito stress assay of KPC cells treated with doxycycline. (G) Immunoblot of orthotopic tumors confirming overexpression of Mfn2 after induction by doxycycline. Data are presented as mean ± SEM.
Supplemental Figure 4. Altered mitochondrial dynamics do not change dihydroorotate dehydrogenase (DHODH) expression.

(A) Leflunomide treatment has no effect on DHODH protein expression. Induced mitochondrial fusion through Drp1 knockout (B) or Mfn2 overexpression (C) shows no change in DHODH protein expression.
Supplemental Figure 5. Leflunomide hinders tumor progression in a heterotopic PDAC mouse model.

(A) Pharmacologically inducing Mfn2 expression with leflunomide suppresses pancreatic cancer cell proliferation. Statistical analysis by 2-way ANOVA. (B) KPC cells treated with 50 µM of Leflunomide exhibit more cell cycle arrest the S phase. ***P < 0.001, ****P < 0.0001 by 2-way ANOVA. (C) TUNEL assay characterizing apoptotic activity of Leflunomide. Scale bar: 20 µm. Leflunomide did not appear to induce apoptosis at a dose of 50 µM when quantified in (D) (*Vehicle control group is shared with Supplemental Figure 2C). (E) Leflunomide (20 mg/kg) treatment slows KPC flank tumor growth, n = 10. ****P < 0.0001 by 2-way ANOVA. (F) Total tumor weight and (G) tumor size of flank tumor after leflunomide treatment or vehicle control, scale bar: 10 mm. Data is significant by unpaired t test. Data are presented as mean ± SEM.
Supplemental Figure 6. Mutational burden in patient-derived pancreatic ductal adenocarcinoma cell lines (PATC).

(A) Genetic annotation of mutations in PATC lines after deep sequencing. KRAS and TP53 represent the largest proportion of oncogenic mutations in human pancreatic cancer cell lines. Quantification of intermediate (B) and tubular (C) mitochondrial morphology in PATC lines from MitoTracker™ staining Figure 5A. Data are presented as mean ± SEM.
**Supplemental Figure 7.** Mitochondrial fusion does not change expression of mitochondrial biogenesis or mtDNA transcription. 

*Ppargc1a* expression in (A) KPC sgDrp1 cells and (B) Mfn2 overexpressing cells with no significant differences observed. (C and D) *Ndufs3* and *Tfam* expression in KPC sgDrp1 cells showed no significant difference compared to sgGFP controls. Data are presented as mean ± SEM.
Full unedited gel for Figure 1A

Drp1

Actin
Full unedited gel for Figure 3A

Mfn2

Actin
Full unedited gel for Figure 4B

43kDa  Actin

250kDa  150kDa  100kDa  75kDa  50kDa  Mfn2
Full unedited gel for Figure 7E

- Mfn2
- CI-Ndufb8
- Actin
Full unedited gel for Supplemental Figure 1G

Drp1

Actin
Full unedited gel for Supplemental Figure 3G

Mfn2

Actin

25kDa
37kDa
50kDa
100kDa
150kDa
250kDa

25kDa
37kDa
50kDa
100kDa
150kDa
250kDa

75kDa

50kDa
37kDa
25kDa

75kDa

50kDa
37kDa
25kDa

Actin
Full unedited gels for Supplemental Figure 4B

DHODH

Tubulin
Full unedited gels for Supplemental Figure 4C

DHODH

Tubulin