**Supplementary Material and Methods and Figure legends**

**Materials and Methods**

**Vector construction**

The sequence of S. cerevisiae NDI1 was amplified from Yeast cDNA using primers,

FWD: 5’ATAAGATCT GCCGCCACC ATGCTATCGAAGAATTTGTAT3’;

REV: 5’TATGAATTCCTACTTATCGTCGTCATCCTTGTAATCTAATCCTTTAAAAAAG

TCTCT3’

The sequence encoding a FLAG-tag was added to the reverse primer. The insert was ligated into pCEFL2-sfi-shuttle vector through restriction sites Bglll and EcoRl. The validated pCEFL2-sfi-shuttle-NDI1-C-FLAG vector was then subcloned into pLenti-CMV-Puro-DEST using the pENTR-sfi1-shuttle Gateway system. (Gateway® LR Clonase® II Enzyme mix).

**Lentiviral vector preparation and infection**

For the production of lentiviruses, a 15 cm dish with 70% confluent 293T cells were transiently co-transfected with VSV-G (7μg), psPAX2 (14μg) packaging plasmids and lentiviral NDI1 construct (21μg). We collected the viral supernatants at 48 and 72 hours after transfection. The supernatants were concentrated by ultracentrifugation at 15,000 g for 4 hours. An empty vector was used as a control. One day before the transduction, cells were plated in tissue culture treated 6-well plates. The next day cells were grown to 50% confluence. Polybrene (10µg/ml) was mixed with concentrated lentiviruses for 30min before adding into each well. After 48 hours of incubation, the transduced cells were then selected with puromycin at 1μg/ml. The cells were continuously maintained at 1μg/ml puromycin to avoid loss of the NDI1 DNA.

**Immunoblot analysis**

Cells were lysed in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40) supplemented with protease inhibitor inhibitors and phosphatase inhibitors from Sigma-Aldrich. The antibodies were from Cell Signaling Technology against S6 (#2217 dilution 1:20,000), phospho-S6 (Ser240/244) (#2211, dilution 1:5000), total AMPK (#2532, dilution 1:5000), phospho-AMPKα (Thr172) (#2535, dilution 1:5000), total AKT(#9272, dilution 1:5000), phospho-AKT(Ser473) (#4060, dilution 1:5000), de-pThr46-4E-BP1 (#4923, dilution 1:1000) phospho-4E-BP1(#9459, dilution 1:3000),total 4E-BP1(#9644 dilution, 1:3000) phos-S6K (#9234, dilution 1:1000), total S6K (#2708, dilution 1:1000), elF4E (#9742, dilution 1:1000), elF4G (#sc-133155), SOX2 (#3579, dilution 1:1000), BMI1 (#6964, 1:5000), ALDH1 (#54135, dilution 1:1000), CD44 (#ab119348, dilution 1:500; Abcam) and GAPDH (#2118 dilution, 1:20,000). OCT3 (SLC22A3) (#ab124826, dilution 1:200, Abcam). Flag-NDI1 was detected using a primary antibody from Sigma (F31665). Secondary horseradish peroxidase-linked goat anti-rabbit, anti-mouse, anti-rat IgG antibodies were obtained from Southern Biotech.

**Cell Viability, Colony formation, and Sphere formation assay**

Cell Viability assay: Cells were grown in 96-well plates and treated with metformin for 72 hours. AlamarBlue was added to cells in culture medium and incubated for 4 hours at 37°C, and fluorescence emission was read at 580–610 nm.

Colony formation assay: the cells were seeded in 6-well plates and treated with metformin. When the control cells developed colonies of ~0.5 mm in diameter, cells were fixed with 1% formaldehyde and stained with crystal violet solution. After washing and drying the plates, serial pictures were taken. Colony number and average area were analyzed using ImageJ.

Sphere formation assay: Cells were seeded in 96-well ultra-low attachment culture dishes (Corning) at 10-100 cells/well with metformin treatment. Medium consisted of serum-free DMEM/F12 Glutamax supplement medium (#10565042), basic fibroblast growth factor (bFGF: 20 ng/ml, #13256029), epithelial growth factor (EGF: 20 ng/ml, #PHG0313), B-27 (1:50 dilution, #17504044), and N2 supplement (1:100 dilution, #17502-048). Ten days after seeding, photos were obtained, and the numbers of sphere colonies on each well were counted using a microscope.

**Flow Cytometry**

CD44 and aldehyde dehydrogenase I (ALDH1) have been introduced as useful markers to identify cancer stem cells (CSCs) (1,2). Cells were stained for viability using BD HorizonTM Fixable Viability Stain 510. Cell surface staining for CD44 was done for 30 minutes at 4 degrees with CD44 (IM7) (BioLegend, San Diego, CA). Intracellular staining for ALDH1 was done using the BD Cytofix/Cytoperm kit and stained with ALDH1. All flow cytometry data acquisition was done using BD LSRFortessa and analyzed using FlowJo software.

**7-methyl GTP pull-down and immunoprecipitation assay**

Cell lysates were incubated with g-Aminophenyl-m7GTP (C10- spacer)-Agarose (Cat # AC-155L; Jena Bioscience) and Protein A Agarose (Cat# 16–125; EMD Millipore) conjugated with elF4G antibody and then washed with lysis buffer. Lysates were then analyzed by western blot using the antibodies described above.

**Insulin and glucose quantification**

Glucose Assay Kit (Cat # ab65333; Abcam) and the Insulin Mouse ELISA Kit (Cat # EMINS; invitrogen) were used to measure the serum glucose and insulin level in control and metformin-treated mice according to the manufacturer’s protocol.

**Gene expression profiling and GSEA analysis**

Sequenced reads were mapped to the reference transcript sequences to compute the transcript abundance. In this process, we used ‘Kallisto'(3) and GRCh38 reference transcript sequences from Ensembl. The expression value for a gene was then set as the maximum transcripts per kilobase million (TPM) of the transcripts mapped to the gene, where Biomart from Ensembl was used to map transcript IDs to gene symbols. The enrichment profiles for gene sets from the Molecular Signatures Database (MsigDB) (4), were computed using single-sample Gene Set Enrichment Analysis (5). Differential gene and gene set expression analyses were performed using the Information Coefficient as a measure of association (6).

**Seahorse assay**

Oxygen consumption rates were measured using a Seahorse XF96 analyzer. Cells were seeded into Seahorse XF96 plates at either 1.5×104 cells/well 24 h before measurements. Respiratory rates were measured in response to sequential injections of oligomycin (2μM), FCCP (sequential additions of 400nM) and rotenone (0.5 μM)/antimycin A (1μM).

**Figure S1:**

The inhibitory effect of metformin on mTOR signaling and cell viability *in vitro* requires functional mitochondrial complex I. **A,** CAL27 (HPV-) and SCC47 (HPV+) were treated with metformin at the indicated dose, and expression of pAMPK/AMPK, pS6/S6, pAKT/AKT, p4E-BP1/4E-BP1,pS6K/S6K were analyzed by western blot. **B,** Viability of HNSCC cells (CAL27 and SCC47) were compared to controls after 72 hours of metformin treatment (3 mM). **C,** Colony formation assay of HNSCC cells (CAL27 and SCC47) treated with metformin (3 mM); colony number and average area were analyzed using ImageJ (\*\*, P<0.01; mean ± SD)

**Figure S2:**

Sphere formation assay of HNSCC cells (CAL27 and SCC47) seeded in 96-well ultra-low attachment plates with DMEM/F12 Glutamax media supplemented with bFGF, EGF, B-27, and N2. Cells were treated with metformin (3 mM) or regular media. Sphere number and average area were analyzed using ImageJ (\*\*, P<0.01; mean ± SD). Size bars are indicated.

**Figure S3:**

This figure shows the same scores as Figure 4 but using the NDI1 expressing tumor samples. The signatures are not significantly enriched in contrast to Fig 5. GSEA analysis reveals the inhibition of the stemness genes sets was not found in the NDI1 expressing tumor samples (NDI1 tumor).

**A,** Plot of Information Coefficient (IC) scores vs. p-values of single-sample GSEA profiles of transcriptional signatures matched against the untreated vs. treated metformin phenotype using the NDI1 expressing tumor samples (NDI1 tumor). **B,** Heatmap showing the individual signatures single-sample GSEA profiles using the NDI1 expressing tumor samples (NDI1 tumor). The bar on top is the untreated vs. treated metformin phenotype, and the numbers on the right are the IC scores and p-values.

**Figure S4:**

Head and neck cancer cells treated with metformin as indicated dose for four days. Cells were collected and stained with fluorescent labeled antibodies for flow cytometry analysis. Shown are representative flow cytometry plots showing the frequency of CD44+ and ALDH1+ cells out of live cells with metformin treatment.

**References**

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