Title: New High-throughput Screen Identifies Compounds That Reduce Viability Specifically In Liver Cancer Cells That Express High Levels of SALL4 by Inhibiting Oxidative Phosphorylation

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Supplemental Materials and Methods:

Antibodies

Western blot antibodies are ACTB from Cell Signaling Technology (4970S), ARG2 from Abcam (ab137069), ATP5D from Abcam (ab97491), ATP5E from Santa Cruz Biotechnology (sc-393695), ATP5G2 from Abcam (ab80325), CASP3 from Cell Signaling Technology (9662), Cleaved CASP3 from Cell Signaling Technology (9661S), MRPL24 from Santa Cruz Biotechnology (sc-393858), NDUFA3 from Abcam (ab68089), SALL4 from Santa Cruz Biotechnology (sc-101147), and SLC25A23 from Santa Cruz Biotechnology (sc-377109). The SALL4 antibody used for immunohistochemistry is from Santa Cruz Biotechnology (sc-101147). The antibody used for immunofluorescence is Cytochrome c from BD Biosciences (556432).

Cell culture

Human hepatocellular carcinoma cell lines SNU-387, SNU-398, SNU-182, SNU-423, SNU-475, SNU-449, and HCC-M, and non small cell lung cancer cell lines H1299 and H661 (ATCC) were grown on standard tissue culture plates in filter sterilized RPMI (Gibco) with 10% heat-inactivated Fetal Bovine Serum (HyClone), 2 mM L-Glutamine (Gibco), and 1% Penicillin-Streptomycin (Gibco). Human hepatocellular carcinoma cell lines HepG2, Hep3B, and Huh-7 (ATCC) are grown on standard tissue culture plates in filter sterilized DMEM (Gibco) with 10% heat-inactivated Fetal Bovine Serum (HyClone), 2 mM L-Glutamine (Gibco), and 1% Penicillin-Streptomycin (Gibco). Human immortalized liver cell line THLE-3 is grown on standard tissue culture plates in filtered BEGM with additives (Lonza), 10% heat-inactivated Fetal Bovine Serum (HyClone), and 1% Penicillin-Streptomycin (Gibco). Cells are incubated at 37°C in a humidified atmosphere of 5% CO_2. Primary HCC cell lines HCC9.2 and HCC26.1 are culture in a media containing Advanced F12/DMEM reduced serum medium (1:1) (Gibco. 12643), 10mM HEPEs (Gibco), 100U/ml Pen /Strep (Gibco), 2mM L-Glutamine (Gibco), 1% N2 (Gibco), 2% B27 (Gibco), 50ng/ml EGF (Millipore), 250ng/ml R-Spondin1 (R&D), and 2µM SB431542 (Tocris). The cells are cultured on standard tissue culture dish coated with 3% matrigel (corning). Cells are incubated at 37°C in a humidified atmosphere of 5% CO_2.

Natural product extract dereplication

Active extracts were subjected to a dereplication procedure as described in the literature[1]. Active fractions were analyzed by accurate MS and MS-MS, and data matched against accurate mass of natural product compounds and A*STAR containing accurate mass and MS/MS mass spectra records of compounds that have been analysed under the same conditions. Oligomycin, 21-hydroxy oligomycin A, leucinostatin A and antimycin A were dereplicated by this method[1].

Fungi Strain F36017 Fermentation (Efrapeptin producing)

F36017 *Tolypocladium niveum* is a soil fungus isolated from United Kingdom. A 7 day old culture of F36017 grown on malt extract agar (Oxoid) was used to prepare 5 flasks of seed cultures, comprising of 50mL of seed medium [yeast extract 4 g/L (BD), malt extract 10g/L (Sigma), glucose 4 g/L (1^{st} Base), pH 5.5] placed in 250 mL Erlenmeyer flasks. These Seed cultures were allowed to grow for 5 days at 24°C with shaking at 200 rpm. At the end of the incubation period, the 5 flasks of seed cultures were combined and homogenized using rotor stator homogenizer (Omni). 5mL of the homogenized seed culture were then used to inoculate each of the 40 flasks containing 6g of vermiculite and 50mL of fermentation medium [maltose 30g/L (Sigma), glucose 10 g/L (1^{st} Base), yeast extract 0.8 g/L (BD), peptone 2 g/L (Oxoid), potassium phosphate monobasic 0.5 g/L (Sigma), magnesium sulphate heptahydrate 0.5 g/L (Merck), ferric chloride 10 mg/L (Sigma), zinc sulphate 2 mg/L (Merck), calcium chloride 55 mg/L (Sigma), pH 6.0]. Static fermentation was carried out for 14 days at 24°C. At the end of the incubation period, the cultures from all 40 flasks were harvested and freeze dried. The dried vermiculite cakes in each flask were scrambled lightly before extracting overnight 2 times with 100 mL methanol per flask. The insoluble materials from each extraction were removed by passing the mixtures through cellulose filter paper (Whatman Grade 4), and the filtrates were dried by rotary evaporation.

Efrapeptin isolation

The culture broths (40× 50 mL, total 2 L) of *Tolypociadium niveum* (F36017 were combined and freeze-dried, partitioned with DCM:MeOH:H_2O 1:1:1. The organic layer was then evaporated to dryness using rotary evaporation. The dried dichloromethane crude extract (0.7 g) was re-dissolved in methanol and separated by C18 reversed-phase preparative HPLC (solvent A: H2O + 0.1% HCOOH, solvent B: ACN + 0.1% HCOOH; flow rate: 30 mL/min, gradient conditions: 70:30 isocratic for 3 minutes; 30% to 40% of solvent B over 12 minutes, 30% to 65% of solvent B over 60 minutes, followed by 65% to 100% of solvent B over 15 minutes, and finally isocratic at 100% of solvent B for 20 minutes) to give 0.6 mg of efrapeptin D (**1,** RT 18.5 min.), 1.0 mg of efrapeptin Eα (**2,** RT 20 min.), 0.5 mg of efrapeptin G (**3,** RT 25min.), and 1.0 mg of efrapeptin H (**4,** RT 27 min.). Efrapeptins were elucidated by comparison accurate mass and ^{1}H NMR data to those of efrapeptins published with activity against bacteria and tumour cells[2].

Drug treatment

Drugs used in the study are PI-103 (Selleckchem), oligomycin A (Selleckchem, LKT Labs), 21-hydroxy oligomycin A (Enzo Life Sciences), oligomycin A, B, and C mix (Enzo Life Sciences), sorafenib tosylate (Selleckchem), bortezomib (Selleckchem), antimycin A (Sigma), cyclosporine A (LC Laboratories), leucinostatin A (BII NPL collection), phenformin (Sigma),

alpelisib (Selleckchem), SB2343 (Selleckchem), idelalisib (Selleckchem), SB2602 (MedKoo Biosciences), CUDC-907 (Selleckchem), and TGX-221 (Selleckchem).

MTT cell viability assay

The MTT assay was used to examine the effect of SALL4 knockdown on isogenic SNU387 cell viability. Three day after viral infection, 3000 SNU-387 cells in a volume of 200 μL were plated into 96-well plates in triplicate, and incubated for the indicated time points. On the day of analysis, 20 μL of MTT solution (5 mg/mL, Sigma) was added, after which the plates were incubated for 2 hours at 37℃ to. After removal of the medium, the purple formazan crystals formed were dissolved in 100 μL DMSO with 10 minute incubation at 37℃. The optical density (OD) of dissolved purple crystal was measured by the Safire 2 plate reader (Tecan) at a wavelength of 570 nm.

CyQUANT cell viability measurements

DNA content of plated cells was measured by application of the CyQUANT Direct Cell Proliferation Kit (Thermo Fisher Scientific) that contains a cell-permeable fluorescent DNA binding dye. Cells were plated in either 96- or 384-well black, clear bottom tissue culture plates (Greiner) and allowed to reach the appropriate confluency before the addition of the appropriate amount of CyQUANT reagent, as detailed in the manufacturer's protocol. Cells were incubated for at least 1 hr at 37°C in a humidified atmosphere of 5% CO_2, after which fluorescence readings were measured by an Infinite M1000 Microplate Reader (Tecan) within a wavelength range of 480-535 nm.

CCK-8 cell viability measurements

Cells were cultured overnight in 96-well plates with 50 μl RPMI 1640 medium (10% FBS) with 1,250 cells per well for SNU-387 Empty Vector and SNU-387 parental cells, and 750 cells per well for SNU387 TgSALL4A and B cells. Cells were grown overnight before drug treatment. Phenformin, at varying concentrations, was dissolved in culture media. 50 μl of the solution was then added to each well. After 96 hr incubation, 10 μl CCK-8 reagent (Dojindo) was added to each well. After 4 hr incubation, optical density values were determined at a wavelength of 450 nm on a SpectraMax M3 Microplate Reader (Molecular Devices).

EdU cell proliferation assay

The Click-iT Plus EdU Alexa Fluor 488 Flow Cytometry Assay (Thermo Fisher Scientific) to assess cell proliferation was carried out following the manufacturer's protocol. SNU-387 isogenic lines were seeded in a 6-well plate overnight, after which the cells were incubated with 10 μM Click-iT EdU for 3 hrs. The cells were harvested and washed with 1% BSA in PBS, and incubated with Click-iT fixative for 15 mins. After fixation, the cells were washed with 1% BSA in PBS and permeabilized in Click-iT saponin-based permeabilization and wash reagent. The click-it reaction was then performed by incubation the cells with Click-iT reaction cocktail for 30 mins to label the EdU-incorporated cells with Alexa Fluor 488 dye. A standard flow cytometry method was used for determining the percentage of S-phase cells in the population using the BD LSR II Cell Analyzer (BD Biosciences).

Cell counts

SNU-387 isogenic cell lines growing at exponential phase were seeded in 6-well plates at a density of 1.5 x 10^5 cells/well. Every 3-4 days, the cells were trypsinized, after which cell

numbers were counted to record the growth of the cells. Then the cells were plated at equal cell number in new plates with fresh medium. Total cell number is presented as viable cells per well after split-adjustment.

SALL4 knockdown by lentiviral transduction

The published lenti shRNA vector pLL3.7 for scrambled (sh-scr), shSALL4-1 and shSALL4-2[3] were transfected into 293FT cells along with packaging plasmid (psPAX2) and envelope plasmid (pMD2.G) using jetPRIME® DNA transfection reagent (Polyplus-transfection® SA) according to the manufacturer's protocol for viral packaging. Viral supernatants were collected twice at 48 hrs and 72 hrs after transfection, and filtered through 0.45 μm sterile filters. Virus stocks were concentrated by ultra-centrifuge at 21,000 g for 2 hrs at 4°C. Viral transduction were carried out using spinoculation. Briefly, fresh medium containing lentivirus and 5 μg/mL Polybrene were added to plated cells. The plates was then centrifuged at 800 g at 37 °C for 1 hr, and incubated at 37°C in a humidified atmosphere of 5% CO_2.

Scrambled:
GGGTACGGTCAGGCAGCTTCTTTCAAGAGAAGAAGCTGCCTGACCGTACCCTTTTTTC
shSALL4-1:
GGCCTTGAAACAAGCCAAGCTATTCAAGAGATAGCTTGGCTTGTTTCAAGGCCTTTTTC
shSALL4-2:
TGCTATTTAGCCAAAGGCAAATTCAAGAGATTTGCCTTTGGCTAAATAGCTTTTTTC

Immunohistochemistry

Immunohistochemistry was performed using Santa Cruz SALL4 antibody (sc-101147). Slides were first deparafinized with xylene, 100% ethanol, 95% ethanol, 70% ethanol and distilled water respectively. After deparafinizing, slides were then blocked for 30 mins in blocking buffer (65 ml 100% methanol, 3.5 ml 30% hydrogen peroxide, 31.5 ml water) to block endogenous peroxidase. Subsequently, antigen retrieval was conducted in 1x pH6 citrate buffer (Sigma Aldrich) and boiled for 30 mins. Slides were washed 3 times with distilled water and blocked in normal blocking serum provided by Vectastain ABC kit for 1 hour in room temperature. Next, slides were then incubated in SALL4 primary antibody diluted 1:400 in blocking serum for 1 hour in room temperature. Prior to staining with secondary antibody, slides were washed 3 times in PBS with 0.1% triton-X. After staining with secondary antibody, slides were incubated in ABC reagent (from Vectastain ABC kit) in a humidified chamber for 1 hour in room temperature following 3 times wash in PBS. Washing was carried out in PBS for 3 times before detection was done using DAB kit (Vector laboratory) and slides were incubated in the dark at room temperature for 5 mins. Lastly, counterstaining was performed in hematoxylin for 15 mins and dehydration in 70% ethanol, 95% ethanol, 100% ethanol and xylene respectively.

Mouse Xenograft

Animals were maintained and studies were carried out according to the Institutional Animal Care and Use Committee protocols. For the SALL4-high models, the SNU-398 cell line and HCC26.1 patient primary cells were cultured as detailed in the aforementioned "Cell culture" methods. *NOD.Cg-Prkdc^{scid} Il2rg^{tm1wjl} SzJ* (NSG) mice, both male and female, were anesthetized

using 2.5% Isofluorane (Sigma). 1,000,000 cells in 200 µl of RPMI/Primary HCC cell media + Matrigel (1:1 ratio) were injected subcutaneously per mouse flank. For the SALL4-low model, the PDX1 tumor was digested with collagenase and dispase, and passed through a 70 µM strainer to obtain a sincle-cell suspension in supplemented DMEM/F12 media. The suspension was treated with red blood cell lysis buffer and DNase. After washing the cells with PBS, the suspension was mixed with an equal volume of Matrigel and injected subcutaneously in the flank of 7 female NSG mice for initial tumor propagation. The 7 PDX1 tumors were harvested after 4 weeks and processed for injection as described previously. Viable cells were counted and mixed with Matrigel to obtain a 2,500,000 cells/ml single-cell suspension. 500,000 PDX1 cells were injected subcutaneously into the left flank of each of 12 NSG mice. Isoflurane was used to anesthetize mice during injections. Drug treatment was carried out when tumors are visible. Drugs were dissolved in vehicle, 5% DMSO (Sigma) and 95% corn oil (Sigma), and injected intraperitoneal at a dose of 20 mg/kg for Sorafenib and 0.1 mg/kg for oligomycin A, with the same doses used in the combination treatment, once daily on weekdays, with no injections on weekends. Mouse weight and tumor size were recorded before each injection. Once tumors reached >1.5 cm in diameter, mice were euthanized and tumors were snap frozen in liquid nitrogen.

Mouse Toxicity Testing

Female NSG mice were injected with vehicle or 0.1 mg/kg of oligomycin A three times a week every Monday, Wednesday, and Friday for 3 weeks, then subjected to the following assays.
(1) Open field test (Locomotor testing): Mice were transported to the procedure room at least two hours prior to experiments to allow for habituation to the novel room. Locomotor activity recordings were carried out using a square open field (40x40cm) in a plexiglass cage, equipped with two rows of photocells sensitive to infrared light. The testing apparatus was enclosed in a ventilated, quiet procedure room. Measurements were performed under low levels of light to minimize stress levels of the mice, and allow for normal exploratory behavior. The mice were introduced into the locomotor cage and allowed to explore freely for 30 mins. Locomotor activity data was collected automatically. The exploratory behaviors were also captured through video recordings. The total distance travelled over 30 mins and the average velocity, from 6 independent measurements, was measured for each mouse.
(2) Grip strength tests: These tests were performed using a grip strength meter. The forelimb and full body grips of each mouse were measured in three successive trials and recorded. Hindlimb measures were calculated using the difference between the grams-force (gF) recorded for the full body and the forelimb. The results of the three tests were averaged for each mouse.
(3) Rotarod test: Mice were placed on the rotor-rod apparatus which linearly accelerated from 4 to 40 rpm at a rate of 0.1 rpm/sec. Mice were tested in four trials, with a 15 minute rest period between tests. The latency to fall and distance travelled by each mouse was recorded.
(4) Home cage recording: Each mouse was monitored in its home cage for 24 hours through video recording, to capture any instances of abnormal neurological events such as seizures.

ChIP-seq analysis

ChIP-seq data were downloaded from NCBI GEO with accession number GSE112729[4]. Reads were mapped by bowtie2 against human reference genome GRCh38. PCR duplicates were removed in the paired-end alignments by samtools rmdup[5]. Peak calling was performed by macs2 with default options. Annotation of the peaks was done by annotatePeaks.pl in Homer

software packages. Alignment files in BAM format were converted to signals by using bedtools[6], and the average coverage of each ChIP-seq experiment was adjusted to 1. bedGraphToBigWig was used to convert the result into bigWig format files. Heatmaps were generated by Deeptools2 along regions on mitochondria genes[7]. Regions were sorted according to the strength of SALL4 signals.

RNA-seq

SALL4-targeting shRNA was transduced into SNU-398 hepatocellular carcinoma (HCC) cell line as previously described[3]. Three days after transduction, the cytoplasm of the cells was removed by dounce homogenizer and nuclear RNA was extracted using the RNeasy Mini Kit (Qiagen). For SNU-387 SALL4A and SALL4B-expressing isogenic cell lines, SNU-387 HCC cells were transduced with SALL4A or SALL4B FUW-Luc-mCh-puro lentiviral constructs[8]. Puromycin was used to select for stable SALL4A or SALL4B-expressing cells. More than two weeks after selection, RNA was harvested from these isogenic cells using RNeasy Mini Kit (Qiagen). The quality of the harvested total RNA was analyzed on Bioanalyzer prior to generation of the sequencing libraries, a RIN value of >9 from all samples were observed. cDNA library construction was then performed using the stranded ScriptSeq Complete Gold kit (Human/Mouse/Rat) (Epicenter; now available through Illumina). Ribosomal RNA depletion was included in the library construction steps. Paired end 76bp sequencing was done using the Illumina HiSeq 2000 sequencer. The paired-end RNA-seq reads were mapped by TopHat2 pipeline against human reference genome GRCh38 with gene annotation GENCODE 24[9]. PCR duplicates were removed in the paired-end alignments by samtools rmdup[5]. Alignments with mapping quality < 20 were also removed. Based on the reads mapped in the transcriptome, gene expression levels in FPKM were determined by cuffdiff in the Cufflinks package[10]. GSEA analysis was preformed following the manual of the GSEA software[11]. Sequencing data has been deposited in the NCBI Gene Expression Omnibus database with accession number GSE114808.

Immunofluorescence assay and image analysis

Cells were plated in 96-well black, clear-bottom plates overnight at 50-80% confluency. The following day, MitoTracker Red CMXRos (300nM, Thermo Fisher Scientific) was added into live cells for 30 minutes at 37°C. Cells were then washed three times for 5 mins in PBS and fixed in 4% PFA for 15 mins at room temperature. Following 3 washes of PBS, cells were then incubated in blocking buffer (5% horse serum, 1% BSA, 0.2% Triton-X in PBS) for 1h at room temperature. Cytochrome-c antibody (BD Pharmigen, clone 6H2.B4) was added at 1:1000 dilution in blocking buffer and incubated overnight at 4°C. The next day, cells were washed three times for 5 mins in PBS and incubated with Alexa-Fluor-488 conjugated anti-mouse antibody (Life Technologies) at 1:400 dilution in blocking buffer for 1h at room temperature. Nuclei were stained with DAPI in blocking buffer. Imaging and quantification of relative intensities of fluorescence signals were performed with the Cytation 5 multi-mode reader and Gen5 software (BioTek).

Targeted mass spectrometry

Samples were re-suspended using 20 µL HPLC grade water for mass spectrometry. 5 µL were injected and analyzed using a hybrid 5500 QTRAP triple quadrupole mass spectrometer (AB/SCIEX) coupled to a Prominence UFLC HPLC system (Shimadzu) via selected reaction monitoring (SRM) of a total of 256 endogenous water soluble metabolites for steady-state

analyses of sample[12]. Some metabolites were targeted in both positive and negative ion mode for a total of 289 SRM transitions using positive/negative ion polarity switching. ESI voltage was +4900 V in positive ion mode and –4500 V in negative ion mode. The dwell time was 3 ms per SRM transition and the total cycle time was 1.55 seconds. Approximately 10-14 data points were acquired per detected metabolite. Samples were delivered to the mass spectrometer via hydrophilic interaction chromatography (HILIC) using a 4.6 mm i.d x 10 cm Amide XBridge column (Waters) at 400 μL/min. Gradients were run starting from 85% buffer B (HPLC grade acetonitrile) to 42% B from 0-5 minutes; 42% B to 0% B from 5-16 minutes; 0% B was held from 16-24 minutes; 0% B to 85% B from 24-25 minutes; 85% B was held for 7 minutes to re-equilibrate the column. Buffer A was comprised of 20 mM ammonium hydroxide/20 mM ammonium acetate (pH=9.0) in 95:5 water:acetonitrile. Peak areas from the total ion current for each metabolite SRM transition were integrated using MultiQuant v2.0 software (AB/SCIEX).

Metabolite profile analyses

Relative intensities of metabolites were normalized to cell number. Metabolite Set Enrichment Analysis (MSEA) was performed on the MetaboAnalyst web server with lists of metabolites with fold change more than or equal to 1.3 either up or down in the isogenic SALL4 expression cell lines compared to empty vector control, with Student's two-tailed t-test p-value of less than 0.05[13].

L-lactate cellular measurements

The L-lactate Assay kit (Abcam) was used to measure cellular lactate levels. 2.2 x 10^6 cells were washed in ice-cold PBS twice, then lysed in 220 μL of assay buffer to achieve a concentration of 10,000 cells per μL. Lysates were then spun down at 13,000 rpm for 5 mins at 4°C to pellet insoluble debris. Soluble fractions were then filtered through >30 kDa centrifugal filter units (Amicon), spun at 14,000 rpm for 20 mins at 4°C, to remove endogenous lactate dehydrogenase subunits (35 kDa) from the lysates. The assay was then performed according to the manufacturer's protocol with 50 μL of lysate (500,000 cells) per well in a 96-well plate, and the inclusion of L-lactate standards to plot a standard curve for lactate quantification.

Oxygen consumption rate and glycolysis stress test measurements

Cells were harvested and plated in the Seahorse XFe96 96-well miniplates (Agilent) coated with collagen. Cell numbers plated were 15,000 for SNU-387, SNU-387 Empty Vector, *Tg:SALL4A* and *Tg:SALL4B* cell lines, 25,000 for SNU-398 and SNU-398 sh-scr cell lines, 35,000 for the SNU-398 shSALL4-1 knockdown cell line, and 40,000 for the SNU-398 shSALL4-2 knockdown cell line. After overnight incubation, cells were washed and media was replaced with the recommended Seahorse Mitostress DMEM media and placed in a CO2-free 37°C incubator for 1 hr. Basal oxygen consumption was then measured by the Seahorse XFe96 Analyzer (Agilent) according to the manufacturer's recommended protocol. The Glycolysis Stress Test was also performed on the isogenic SALL4 expressing cell lines, prepared as described above, according to the manufacturer's recommended protocol. Cells were also subjected to the CyQUANT DNA quantification assay (Thermo Fisher Scientific) to measure DNA content, serving as a basis to normalize oxygen consumption rates with respect to cell number.

RNA/DNA extraction & quantitative RT-PCR analysis

RNA isolation was performed using the RNeasy Plus Mini Kit (Qiagen). Genomic/mitochondrial DNA isolation was performed using the QIAamp DNA Mini Kit (Qiagen). cDNA was synthesized from purified RNA with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative PCR for cDNA or genomic/mitochondrial DNA was performed on the ViiA 7 Real-Time PCR system (ThermoFisher Scientific) using the PowerUP SYBR Green Master Mix (Applied Biosystems). The ΔΔCt method was used for relative quantification. RT-PCR primers are:
18S rRNA forward: 5'- GTAACCCGTTGAACCCCATT -3'
18S rRNA reverse: 5'- CCATCCAATCGGTAGTAGCG -3'
ACTB forward: 5'- CAGAGCCTCGCCTTTGCCGATC -3'
ACTB reverse: 5'- CATCCATGGTGAGCTGGCGGCG -3'
ARG2 forward: 5'- CGCGAGTGCATTCCATCCT -3'
ARG2 reverse: 5'- TCCAAAGTCTTTTAGGTGGCAG -3'
B2M forward: 5'- CACTGAAAAAGATGAGTATGCC -3'
B2M reverse: 5'- AACATTCCCTGACAATCCC -3'
CLYBL forward: 5'- TCCCCAGACTTGGATATAGTTCC -3'
CLYBL reverse: 5'- TGCACAATCTACATTCAGGGATG -3'
MinorArc forward: 5'- CTAAATAGCCCACACGTTCCC -3'
MinorArc reverse: 5'- AGAGCTCCCGTGAGTGGTTA -3'
MRPL24 forward: 5'- GCCAGGTCAAACTTGTGGAT -3'
MRPL24 reverse: 5'- CCCTGATCGTGTGGAGACTC -3'
ND1 forward: 5'- ACGCCATAAAACTCTTCACCAAAG -3'
ND1 reverse: 5'- GGGTTCATAGTAGAAGAGCGATGG -3'
ND4 forward: 5'- ACCTTGGCTATCATCACCCGAT -3'
ND4 reverse: 5'- AGTGCGATGAGTAGGGGAAGG -3'
NRF1 forward: 5'- AGGAACACGGAGTGACCCAA -3'
NRF1 reverse: 5'- TGCATGTGCTTCTATGGTAGC -3'
NRF2 forward: 5'- AAGTGACAAGATGGGCTGCT -3'
NRF2 reverse: 5'- TGGACCACTGTATGGGATCA -3'
PGC-1α forward: 5'- CAAGCCAAACCAACAACTTTATCTCT -3'
PGC-1α reverse: 5'- CACACTTAAGGTGCGTTCAATAGTC -3'
PGC-1β forward: 5'- GGCAGGTTCAACCCCGA -3'
PGC-1β reverse: 5'- CTTGCTAACATCACAGAGGATATCTTG -3'
SALL4 forward: 5'- GCGAGCTTTTACCACCAAAG -3'
SALL4 reverse: 5'- CACAACAGGGTCCACATTCA -3'
SALL4A forward: 5'- TCCCCAGACTTGGATATAGTTCC -3'
SALL4A reverse: 5'- TGCACAATCTACATTCAGGGATG -3'
SALL4B forward: 5'- GGTGGATGTCAAACCCAAAG -3'
SALL4B reverse: 5'- ATGTGCCAGGAACTTCAACC
SLC25A10 forward: 5'- GTGTCGCGCTGGTACTTC -3'
SLC25A10 reverse: 5'- CACCTCCTGCTGCGTCTG -3'
SUMO1 forward: 5'- TTGGAACACCCTGTCTTTGAC -3'
SUMO1 reverse: 5'- ACCGTCATCATGTCTGACCA -3'
TFAM forward: 5'- CCGAGGTGGTTTTCATCTGT -3'
TFAM reverse: 5'- ACGCTGGGCAATTCTTCTAA -3'

Supplemental Figures/Tables:

Fig. S1. SALL4 isogenic cell lines are dependent on SALL4 for cell viability. (**A**) *SALL4* mRNA expression in *SALL4* endogenous cell lines used in the screen, measured by qRT-PCR and normalized to *ACTB* (mean of 4 replicates ± SD). (**B**) *SALL4* mRNA expression in SNU-387 isogenic empty vector, *SALL4A*, and *SALL4B* expressing cell lines used in the screen, measured by qRT-PCR and normalized to *ACTB* (mean of 2 replicates ± SD). (**C**) Western blot of SALL4 protein in the *SALL4* endogenous cell lines, with ACTB loading control. Bands were quantified by densitometry with SNU-387 bands as reference. (**D**) Western blot of SALL4 protein isoforms and *SALL4* knockdown validation in the isogenic cell lines, with ACTB loading control. Bands were quantified by densitometry with sh-scr bands as reference. (**E**) MTT oxidoreductase-dependent cell viability assay on *SALL4* isogenic cell lines with *SALL4* knockdown, normalized to day 5 sh-scr scrambled control (mean of 3 replicates ± SD). (**F**) Cell counts of SALL4-expressing isogenic cell lines over 10 days (mean of 3 replicates ± SD). (**G**) EdU incorporation, during DNA synthesis, measurements for the percentage of EdU labeled cells after 3 hrs of treatment for the SALL4-expressing isogenic cell lines (performed in singlet).

Fig. S2. Natural product and small molecule screening hits. (**A**) Cell viability fold change plots of control compounds obtained from the pilot screen and used for the complete screen, measured with CellTiter-Glo cell viability reagent, and normalized to DMSO-treated cell viability (mean of 3 replicates ± SD). (**B**) Cell viability dose-response curves for cells treated for 96 hrs with synthetic compound hit PI-103, measured with CellTiter-Glo and CyQUANT reagents and normalized to untreated cell viability (mean of 3 replicates ± SD). (**C**) Cell viability dose-response curves for cells treated for 96 hrs with hit compounds from the natural product extract screen, oligomycin, efrapeptin, antimycin, and leucinostatin, measured with CyQUANT reagent and normalized to untreated cell viability (mean of 3 replicates ± SD). (**D**) Western blot for apoptosis marker cleaved caspase-3 and control total caspase-3 protein levels in oligomycin A-treated SNU-398 cells. Bands were quantified by densitometry with DMSO bands as reference.

Fig. S3. Oligomycin A suppresses SALL4-dependent tumorigenesis. (**A**) *SALL4* mRNA expression in HCC cell lines with respect to immortalized normal liver cell line THLE-3 *SALL4* transcript levels, measured by qRT-PCR and normalized to *18S* rRNA (mean of 3 replicates ± SD). Oligomycin A IC_{50} values from dose response curves in Fig. 3A are detailed above the bar graphs for corresponding cell lines. (**B**) *SALL4* mRNA expression in a pair of $SALL4^{hi}$ and $SALL4^{lo}$ NSCLC cell lines with respect to immortalized normal liver cell line THLE-3 *SALL4* transcript levels, measured by qRT-PCR and normalized to *18S* rRNA (mean of 2 replicates ± SD). Oligomycin A IC_{50} values from dose response curves in Fig. S3C are detailed above the bar graphs for corresponding cell lines. (**C**) Cell viability dose-response curves for lung cancer cell lines in (**B**) treated with oligomycin A, measured with CellTiter-Glo reagent and normalized to untreated cell viability (mean of 3 replicates ± SD). (**D**) Tumor images from the SNU-398 mouse xenograft experiment in Fig. 3B. (**D**) Tumor images from the SNU-398 mouse xenograft experiment in Fig. 3C. (**E**) Tumor images from the HCC26.1 mouse patient-derived xenograft experiment in Fig. 3E. (**F**) SALL4 immunohistochemistry on a PDX1 tumor section and a SALL4 positive control tumor section. (**G**) Tumor images from the PDX1 mouse patient-derived xenograft experiment in Fig. 3G. Four tumors were excised on day 32 as their size reached the designated animal protocol endpoint while the remaining mice continued drug treatment till day 36, when all remaining tumors reached the endpoint. (**H**) Open field test conducted on mice injected with vehicle (n=6) and 0.1 mg/kg oligomycin A (n=6) over 3 weeks (mean ± SD). (**I**) Grip strength test conducted on the mice in (**H**) (mean ± SD). (**J**) Rotarod test conducted on the mice in (**H**) (mean ± SD). (**K**) HCC patient stratification by SALL4 expression and diabetics. Numbers above bar graphs indicate absolute patient numbers. (**L**) Cell viability dose-response curves for cells treated for 96 hrs with phenformin or oligomycin A, measured with CCK-8 dehydrogenase activity assay and normalized to untreated cell viability (mean of 3 replicates ± SD).

Fig. S4. SALL4 expression upregulates oxidative phosphorylation gene expression. (**A**) RNA-seq expression level fold change for SALL4, in the SNU-398 *SALL4* knockdown and isogenic *SALL4* expressing cell lines, normalized respectively to expression levels in the SNU-398 input and SNU-387 empty vector control cell line, performed in singlet. (**B**) RNA-seq expression level fold change for a panel of mitochondrial genes from Fig. 4D with *SALL4* knockdown in the SNU-398 cells, normalized to expression levels in the SNU-398 control, performed in singlet. (**C**) mRNA expression validation of selected mitochondrial genes in the *SALL4* expressing isogenic cell lines used in the screen, measured by qRT-PCR and normalized to *18S* rRNA (mean of 3 replicates ± SD). (**D**) mRNA expression validation of the mitochondrial genes from (**C**) with *SALL4* knockdown for 72 hrs in the SNU-398 cell line, measured by qRT-PCR and normalized to *18S* rRNA (mean of 2 replicates ± SD). (**E**) GSEA plots for oxidative phosphorylation from analysis of the RNA-seq data set in (**A**). (**F**) Western blots for SALL4-bound mitochondrial genes and ACTB loading control in the cell lines used in the screen. Bands were quantified by densitometry with SNU-387 and EV bands as references. (**G**) Western blots for the genes in (**F**) in the SNU-398 cell line 72 hours after *SALL4* knockdown. Bands were quantified by densitometry with sh-scr bands as reference.

Fig. S5. Oxidative phosphorylation and glycolysis metabolite changes induced by SALL expression. (**A**) Metabolite Set Enrichment Analysis (MSEA) of significantly altered metabolites (1.3 fold change, $P < 0.05$) in the SNU-387 *Tg:SALL4A* cells compared to empty

vector control. (**B**) MSEA of significantly altered metabolites (1.3 fold change, $P < 0.05$) in the SNU-387 *Tg:SALL4B* cells compared to empty vector control. (**C**) Fold change of malate-aspartate shuttle metabolites in the SALL4-expressing isogenic lines normalized to empty vector control (mean of 3 replicates ± SD). (**D**) Fold change of glycolytic metabolites in the SALL4-expressing isogenic lines normalized to empty vector control (mean of 3 replicates ± SD). (**E**) L-lactate measurements, utilizing a lactate dehydrogenase enzymatic assay, in the SALL4 isogenic cell lines and no enzyme controls, normalized by cell number (mean of 2 replicates ± SD). (**F**) Extracellular acidification rate (ECAR) measurements per DNA content in the SALL4 isogenic lines, normalized to CyQUANT DNA quantification reagent values (mean of 3 replicates ± SD). (**G**) Glycolysis stress test assessing ECAR when cells are treated with glucose post starvation, ATP synthase inhibitor oligomycin, and glycolysis inhibitor 2-Deoxy-D-glucose that quantifies glycolytic flux and glycolytic capacity, performed on the SALL4-expressing isogenic lines (mean of 3 replicates ± SD).

Fig. S6. PI3K and mTOR inhibitor have limited selectivity for SALL4 expressing cells. (**A**) Cell viability dose-response curves for cells treated for 72 hrs with selective PI3K or mTOR inhibitors alpelisib, SB2343, idelalisib, SB2602, CUDC-907, and TGX-221 measured with CellTiter-Glo reagent and normalized to DMSO-treated cell viability (mean of 3 replicates ± SD).

Fig. S7. SALL4 does not directly regulate the Urea cycle and increases mtDNA copy number. (**A**) Fold change of urea cycle metabolites in the SALL4-expressing isogenic lines normalized to empty vector control (mean of 3 replicates ± SD). (**B**) Representative ChIP-seq input, H3K27ac, and SALL4 peaks for urea cycle genes. (**C**) mtDNA quantification with primers to the Minor Arc, *ND1* and *ND4* genes in *SALL4* endogenous and isogenic cell lines used in the screen, measured by qRT-PCR and normalized to *B2M* (mean of 3 replicates ± SD). (**D**) mRNA expression of mitochondrial biogenesis genes in the *SALL4* expressing isogenic cell lines used in the screen, measured by qRT-PCR and normalized to *18S* rRNA (mean of 3 replicates ± SD). (**E**) Representative ChIP-seq input, H3K27ac, and SALL4 peaks for the mitochondrial biogenesis genes in (**D**).

Table S1. Oxidative phosphorylation inhibitors are potent and selective against SALL4-expressing cancer cells. (**A**) Summary of IC_{50} and selectivity values for oxidative phosphorylation inhibitors tested in the SALL4 endogenous HCC cell lines used in the screen. (**B**) Summary of IC_{50} and selectivity values for oxidative phosphorylation inhibitors tested in the SALL4 endogenous NSCLC cell line pair in Fig. S3C.

Table S2. SALL4 binds a significant number of mitochondrial genes. (**A**) List of mitochondrial genes bound by SALL4 from previously published SNU-398 ChIP-seq experiments.

Table S3. SALL4 upregulates oxidative phosphorylation gene expression by RNA-seq GSEA. (**A**) Gene sets upregulated in the SNU-398 input sample compared to SNU-398 shSALL4-1 knockdown. (**B**) Gene sets upregulated in the SNU-398 shSALL4-1 knockdown sample compared to SNU-398 input. (**C**) Gene sets upregulated in the SNU-387 Empty Vector cell line compared to SNU-387 *Tg:SALL4A*. (**D**) Gene sets upregulated in the SNU-387

Tg:SALL4A cell line compared to SNU-387 Empty Vector. (**E**) Gene sets upregulated in the SNU-387 Empty Vector cell line compared to SNU-387 *Tg:SALL4B*. (**F**) Gene sets upregulated in the SNU-387 *Tg:SALL4B* cell line compared to SNU-387 Empty Vector.

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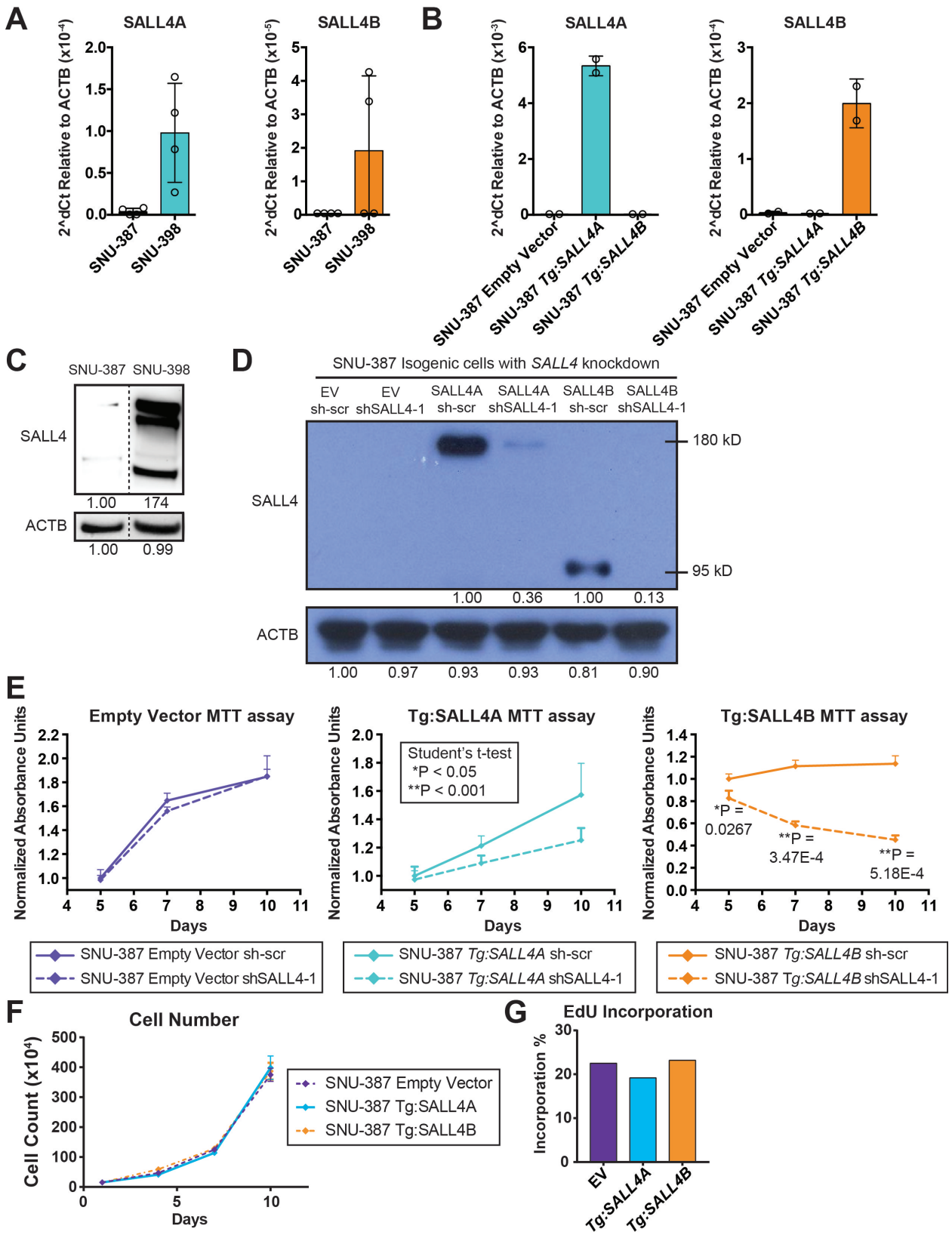
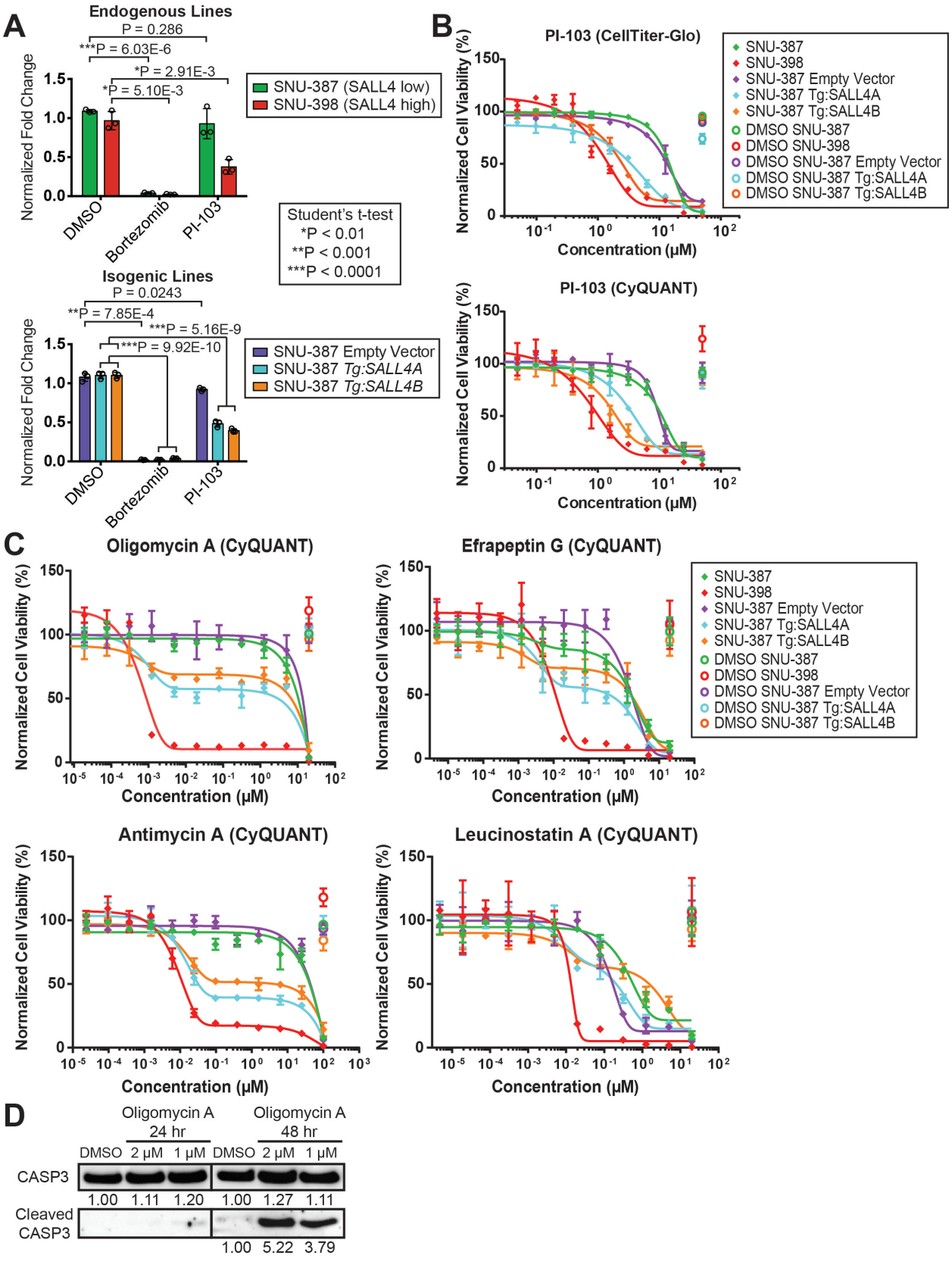
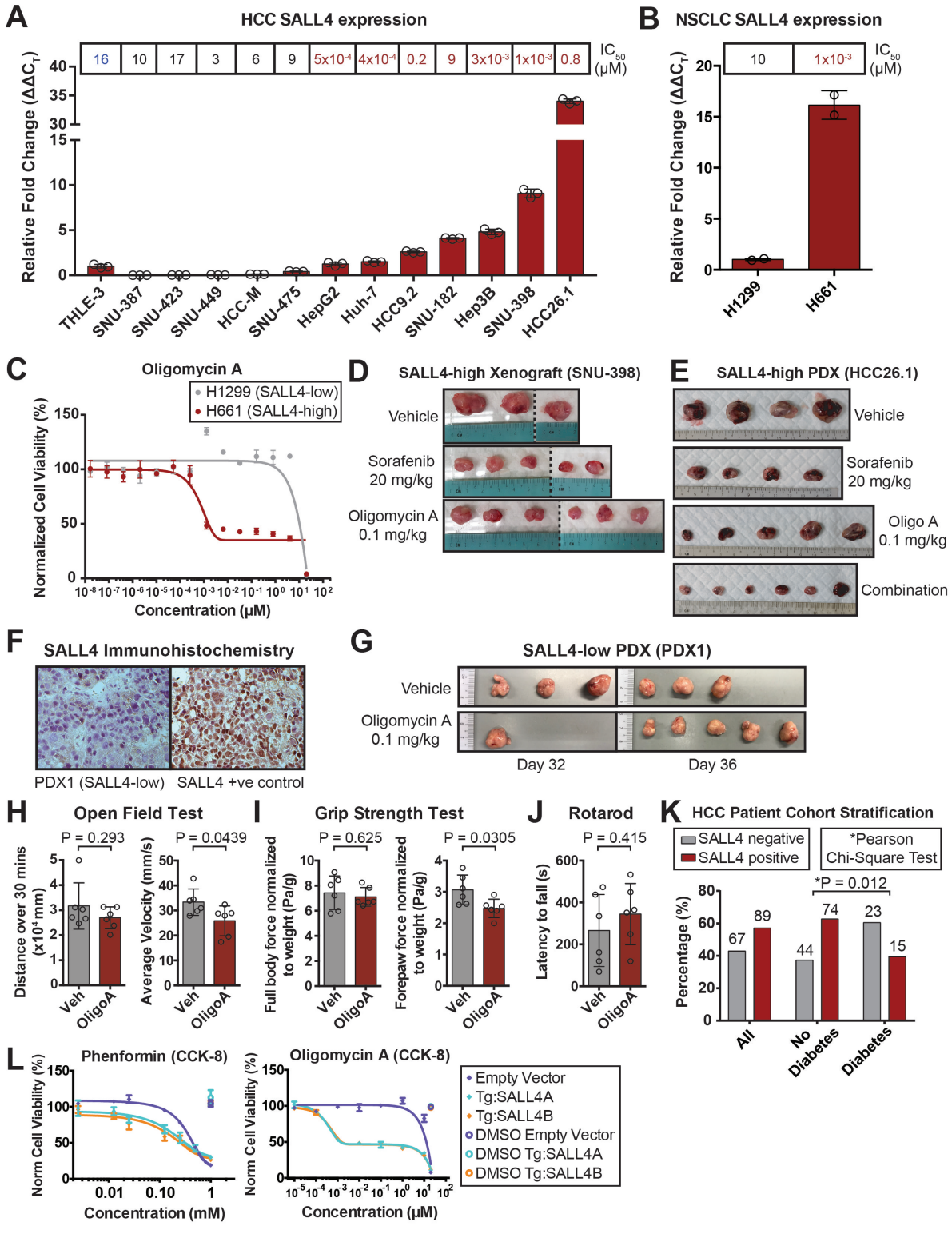
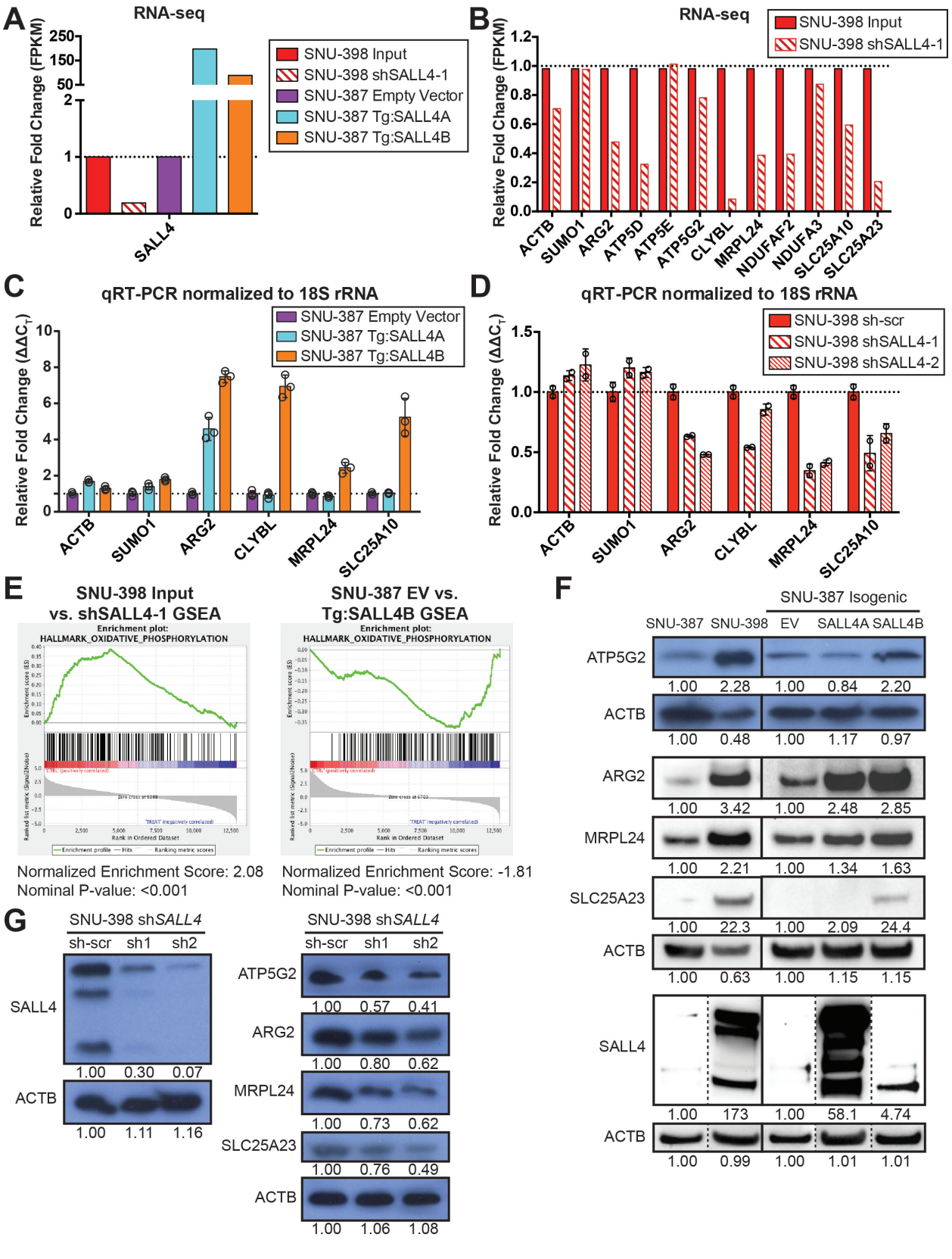
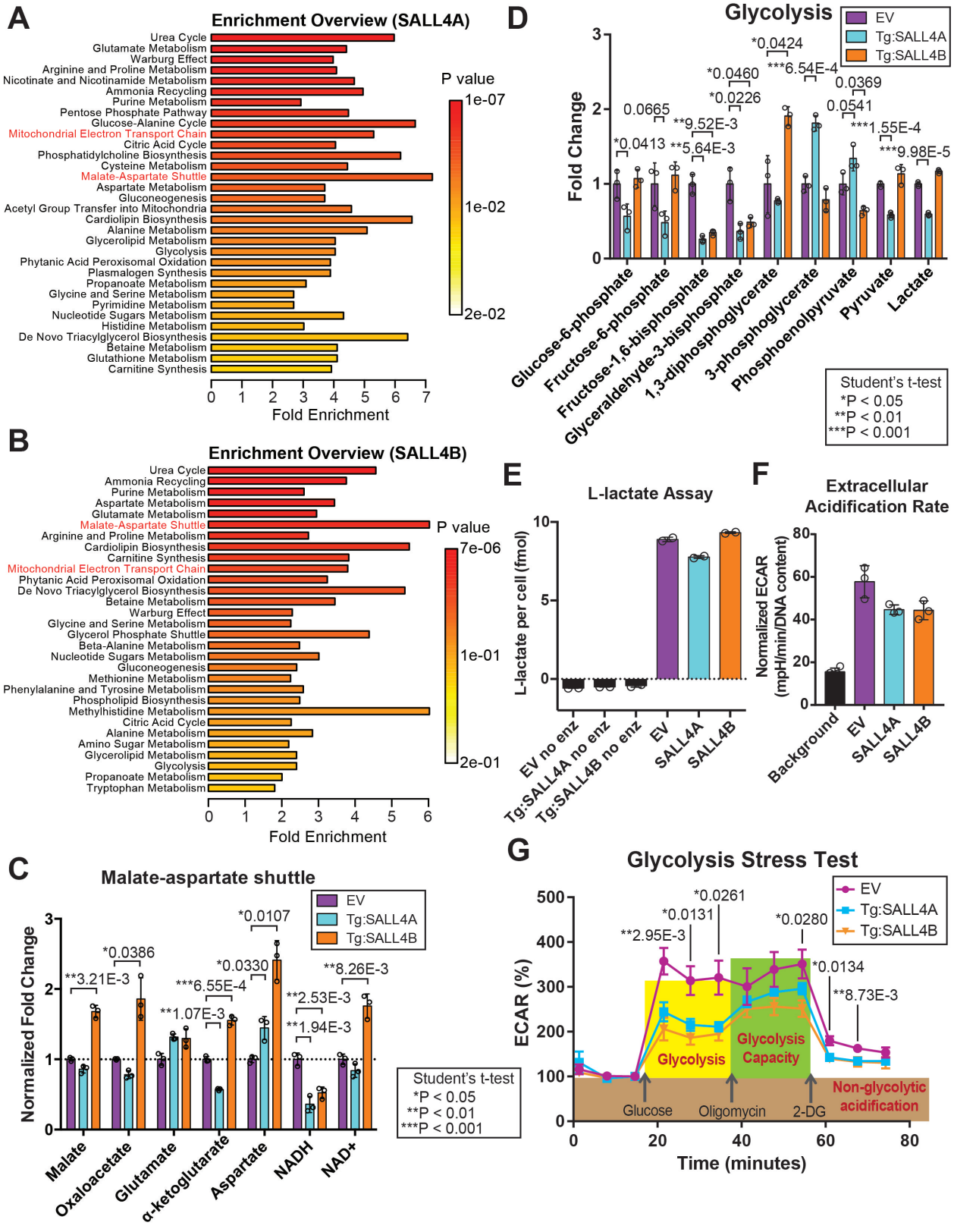
Author names in bold designate shared co-first authorship.

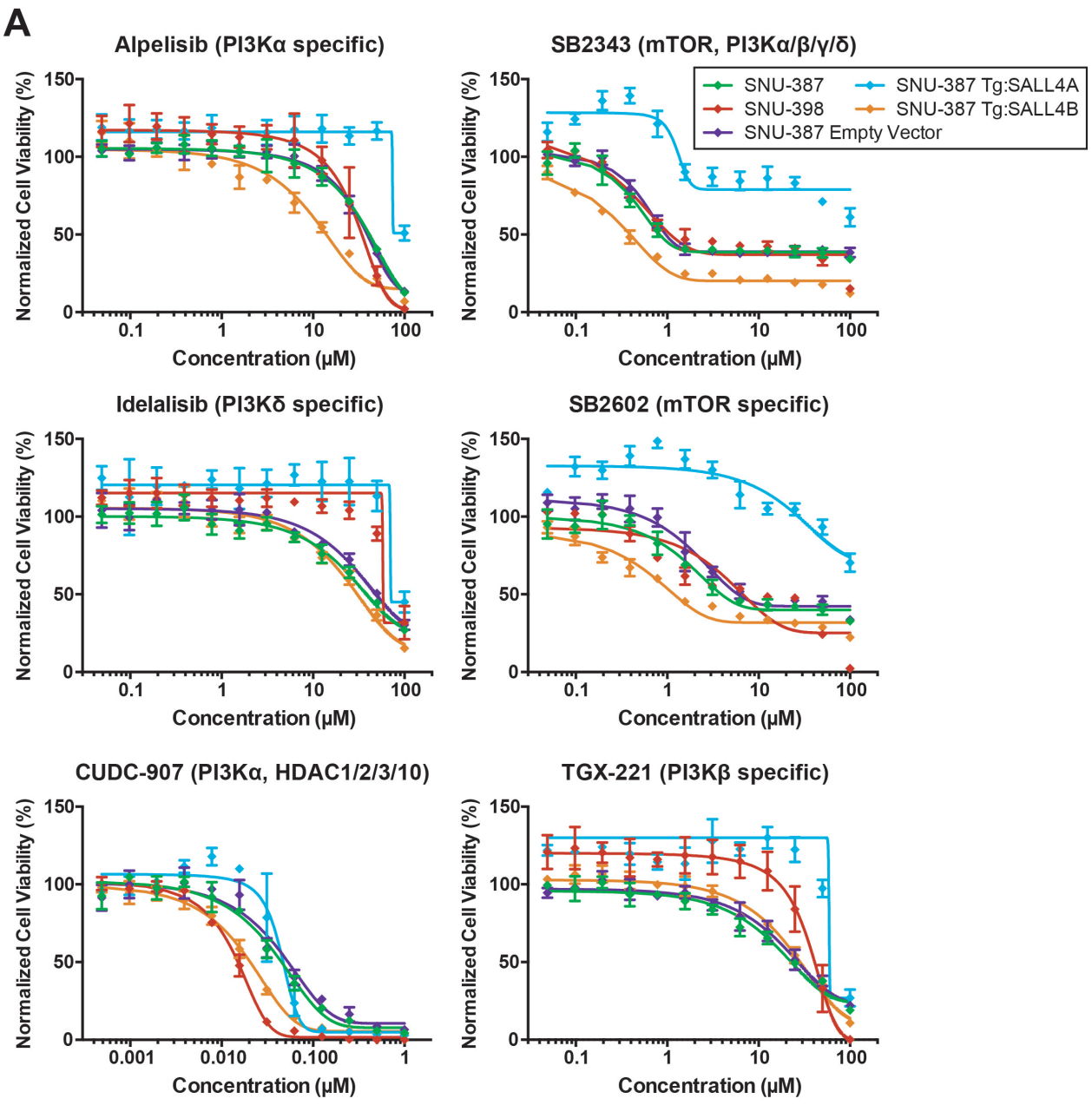

A
SALL4A
2^-dCt Relative to ACTB (x10^-4)
SNU-387
SNU-398
SALL4B
2^-dCt Relative to ACTB (x10^-5)
B
SALL4A
2^-dCt Relative to ACTB (x10^-3)
SNU-387 Empty Vector
SNU-387 Tg:SALL4A
SNU-387 Tg:SALL4B
SALL4B
2^-dCt Relative to ACTB (x10^-4)
C
SNU-387 SNU-398
SALL4
1.00 174
ACTB
1.00 0.99
D
SNU-387 Isogenic cells with SALL4 knockdown
EV sh-scr
EV shSALL4-1
SALL4A sh-scr
SALL4A shSALL4-1
SALL4B sh-scr
SALL4B shSALL4-1
SALL4
180 kD
95 kD
1.00 0.36 1.00 0.13
ACTB
1.00 0.97 0.93 0.93 0.81 0.90
E
Empty Vector MTT assay
Tg:SALL4A MTT assay
Tg:SALL4B MTT assay
Normalized Absorbance Units
Days
Student's t-test
*P < 0.05
**P < 0.001
*P = 0.0267
**P = 3.47E-4
**P = 5.18E-4
SNU-387 Empty Vector sh-scr
SNU-387 Empty Vector shSALL4-1
SNU-387 Tg:SALL4A sh-scr
SNU-387 Tg:SALL4A shSALL4-1
SNU-387 Tg:SALL4B sh-scr
SNU-387 Tg:SALL4B shSALL4-1
F
Cell Number
Cell Count (x10^4)
Days
SNU-387 Empty Vector
SNU-387 Tg:SALL4A
SNU-387 Tg:SALL4B
G
EdU Incorporation
Incorporation %
EV
Tg:SALL4A
Tg:SALL4B

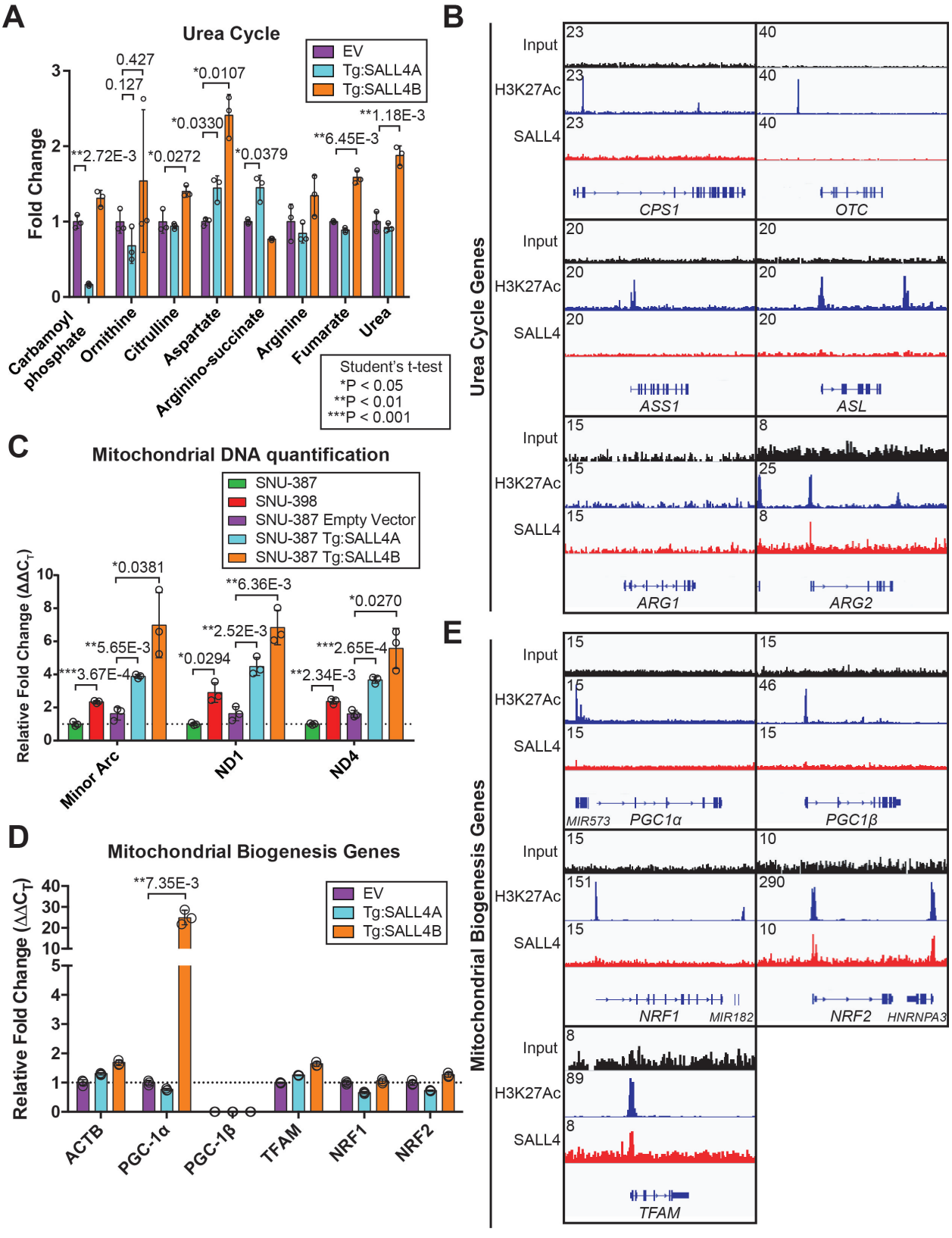

A
Endogenous Lines
P = 0.286
***P = 6.03E-6
*P = 2.91E-3
*P = 5.10E-3
Normalized Fold Change
SNU-387 (SALL4 low)
SNU-398 (SALL4 high)
DMSO
Bortezomib
PI-103
Student's t-test
*P < 0.01
**P < 0.001
***P < 0.0001
Isogenic Lines
P = 0.0243
**P = 7.85E-4
***P = 5.16E-9
***P = 9.92E-10
SNU-387 Empty Vector
SNU-387 Tg:SALL4A
SNU-387 Tg:SALL4B
B
PI-103 (CellTiter-Glo)
Normalized Cell Viability (%)
Concentration (μM)
SNU-387
SNU-398
SNU-387 Empty Vector
SNU-387 Tg:SALL4A
SNU-387 Tg:SALL4B
DMSO SNU-387
DMSO SNU-398
DMSO SNU-387 Empty Vector
DMSO SNU-387 Tg:SALL4A
DMSO SNU-387 Tg:SALL4B
PI-103 (CyQUANT)
C
Oligomycin A (CyQUANT)
Efrapeptin G (CyQUANT)
Antimycin A (CyQUANT)
Leucinostatin A (CyQUANT)
D
Oligomycin A
24 hr
48 hr
DMSO 2 μM 1 μM DMSO 2 μM 1 μM
CASP3
1.00 1.11 1.20 1.00 1.27 1.11
Cleaved CASP3
1.00 5.22 3.79


A
HCC SALL4 expression
IC50 (μM)
16
10
17
3
6
9
5x10-4
4x10-4
0.2
9
3x10-3
1x10-3
0.8
Relative Fold Change (ΔΔCT)
THLE-3
SNU-387
SNU-423
SNU-449
HCC-M
SNU-475
HepG2
Huh-7
HCC9.2
SNU-182
Hep3B
SNU-398
HCC26.1
B
NSCLC SALL4 expression
10
1x10-3
H1299
H661
C
Oligomycin A
H1299 (SALL4-low)
H661 (SALL4-high)
Normalized Cell Viability (%)
Concentration (μM)
D
SALL4-high Xenograft (SNU-398)
Vehicle
Sorafenib 20 mg/kg
Oligomycin A 0.1 mg/kg
E
SALL4-high PDX (HCC26.1)
Vehicle
Sorafenib 20 mg/kg
Oligo A 0.1 mg/kg
Combination
F
SALL4 Immunohistochemistry
PDX1 (SALL4-low)
SALL4 +ve control
G
SALL4-low PDX (PDX1)
Vehicle
Oligomycin A 0.1 mg/kg
Day 32
Day 36
H
Open Field Test
P = 0.293
P = 0.0439
Distance over 30 mins (x10⁴ mm)
Average Velocity (mm/s)
Veh
OligoA
I
Grip Strength Test
P = 0.625
P = 0.0305
Full body force normalized to weight (Pa/g)
Forepaw force normalized to weight (Pa/g)
J
Rotarod
P = 0.415
Latency to fall (s)
K
HCC Patient Cohort Stratification
SALL4 negative
SALL4 positive
*Pearson Chi-Square Test
*P = 0.012
Percentage (%)
67
89
44
74
23
15
All
No Diabetes
Diabetes
L
Phenformin (CCK-8)
Oligomycin A (CCK-8)
Norm Cell Viability (%)
Concentration (mM)
Concentration (μM)
Empty Vector
Tg:SALL4A
Tg:SALL4B
DMSO Empty Vector
DMSO Tg:SALL4A
DMSO Tg:SALL4B


A
RNA-seq
Relative Fold Change (FPKM)
SALL4
SNU-398 Input
SNU-398 shSALL4-1
SNU-387 Empty Vector
SNU-387 Tg:SALL4A
SNU-387 Tg:SALL4B
B
RNA-seq
SNU-398 Input
SNU-398 shSALL4-1
Relative Fold Change (FPKM)
ACTB
SUMO1
ARG2
ATP5D
ATP5E
ATP5G2
CLYBL
MRPL24
NDUFAF2
NDUFA3
SLC25A10
SLC25A23
C
qRT-PCR normalized to 18S rRNA
SNU-387 Empty Vector
SNU-387 Tg:SALL4A
SNU-387 Tg:SALL4B
Relative Fold Change (ΔΔC$_T$)
ACTB
SUMO1
ARG2
CLYBL
MRPL24
SLC25A10
D
qRT-PCR normalized to 18S rRNA
SNU-398 sh-scr
SNU-398 shSALL4-1
SNU-398 shSALL4-2
Relative Fold Change (ΔΔC$_T$)
ACTB
SUMO1
ARG2
CLYBL
MRPL24
SLC25A10
E
SNU-398 Input
vs. shSALL4-1 GSEA
Enrichment plot:
HALLMARK_OXIDATIVE_PHOSPHORYLATION
Normalized Enrichment Score: 2.08
Nominal P-value: <0.001
SNU-387 EV vs.
Tg:SALL4B GSEA
Enrichment plot:
HALLMARK_OXIDATIVE_PHOSPHORYLATION
Normalized Enrichment Score: -1.81
Nominal P-value: <0.001
F
SNU-387 Isogenic
SNU-387 SNU-398 EV SALL4A SALL4B
ATP5G2
1.00 2.28 1.00 0.84 2.20
ACTB
1.00 0.48 1.00 1.17 0.97
ARG2
1.00 3.42 1.00 2.48 2.85
MRPL24
1.00 2.21 1.00 1.34 1.63
SLC25A23
1.00 22.3 1.00 2.09 24.4
ACTB
1.00 0.63 1.00 1.15 1.15
SALL4
1.00 173 1.00 58.1 4.74
ACTB
1.00 0.99 1.00 1.01 1.01
G
SNU-398 shSALL4
sh-scr sh1 sh2
SALL4
1.00 0.30 0.07
ACTB
1.00 1.11 1.16
SNU-398 shSALL4
sh-scr sh1 sh2
ATP5G2
1.00 0.57 0.41
ARG2
1.00 0.80 0.62
MRPL24
1.00 0.73 0.62
SLC25A23
1.00 0.76 0.49
ACTB
1.00 1.06 1.08


A
Enrichment Overview (SALL4A)
Urea Cycle
Glutamate Metabolism
Warburg Effect
Arginine and Proline Metabolism
Nicotinate and Nicotinamide Metabolism
Ammonia Recycling
Purine Metabolism
Pentose Phosphate Pathway
Glucose-Alanine Cycle
Mitochondrial Electron Transport Chain
Citric Acid Cycle
Phosphatidylcholine Biosynthesis
Cysteine Metabolism
Malate-Aspartate Shuttle
Aspartate Metabolism
Gluconeogenesis
Acetyl Group Transfer into Mitochondria
Cardiolipin Biosynthesis
Alanine Metabolism
Glycerolipid Metabolism
Glycolysis
Phytanic Acid Peroxisomal Oxidation
Plasmalogen Synthesis
Propanoate Metabolism
Glycine and Serine Metabolism
Pyrimidine Metabolism
Nucleotide Sugars Metabolism
Histidine Metabolism
De Novo Triacylglycerol Biosynthesis
Betaine Metabolism
Glutathione Metabolism
Carnitine Synthesis
P value
1e-07
1e-02
2e-02
0 1 2 3 4 5 6 7
Fold Enrichment
B
Enrichment Overview (SALL4B)
Urea Cycle
Ammonia Recycling
Purine Metabolism
Aspartate Metabolism
Glutamate Metabolism
Malate-Aspartate Shuttle
Arginine and Proline Metabolism
Cardiolipin Biosynthesis
Carnitine Synthesis
Mitochondrial Electron Transport Chain
Phytanic Acid Peroxisomal Oxidation
De Novo Triacylglycerol Biosynthesis
Betaine Metabolism
Warburg Effect
Glycine and Serine Metabolism
Glycerol Phosphate Shuttle
Beta-Alanine Metabolism
Nucleotide Sugars Metabolism
Gluconeogenesis
Methionine Metabolism
Phenylalanine and Tyrosine Metabolism
Phospholipid Biosynthesis
Methylhistidine Metabolism
Citric Acid Cycle
Alanine Metabolism
Amino Sugar Metabolism
Glycerolipid Metabolism
Glycolysis
Propanoate Metabolism
Tryptophan Metabolism
P value
7e-06
1e-01
2e-01
0 1 2 3 4 5 6
Fold Enrichment
C
Malate-aspartate shuttle
EV
Tg:SALL4A
Tg:SALL4B
Normalized Fold Change
**3.21E-3
*0.0386
**1.07E-3
***6.55E-4
*0.0330
*0.0107
**1.94E-3
**2.53E-3
**8.26E-3
Malate
Oxaloacetate
Glutamate
α-ketoglutarate
Aspartate
NADH
NAD+
Student's t-test
*P < 0.05
**P < 0.01
***P < 0.001
D
Glycolysis
EV
Tg:SALL4A
Tg:SALL4B
Fold Change
*0.0413
0.0665
**5.64E-3
**9.52E-3
*0.0460
*0.0226
*0.0424
***6.54E-4
0.0369
0.0541
***1.55E-4
***9.98E-5
Glucose-6-phosphate
Fructose-6-phosphate
Fructose-1,6-bisphosphate
Glyceraldehyde-3-bisphosphate
1,3-diphosphoglycerate
3-phosphoglycerate
Phosphoenolpyruvate
Pyruvate
Lactate
Student's t-test
*P < 0.05
**P < 0.01
***P < 0.001
E
L-lactate Assay
L-lactate per cell (fmol)
EV no enz
Tg:SALL4A no enz
Tg:SALL4B no enz
EV
SALL4A
SALL4B
F
Extracellular Acidification Rate
Normalized ECAR (mpH/min/DNA content)
Background
EV
SALL4A
SALL4B
G
Glycolysis Stress Test
EV
Tg:SALL4A
Tg:SALL4B
ECAR (%)
**2.95E-3
*0.0131
*0.0261
*0.0280
*0.0134
**8.73E-3
Glycolysis
Glycolysis Capacity
Non-glycolytic acidification
Glucose
Oligomycin
2-DG
Time (minutes)


A
Alpelisib (PI3Kα specific)
SB2343 (mTOR, PI3Kα/β/γ/δ)
Idelalisib (PI3Kδ specific)
SB2602 (mTOR specific)
CUDC-907 (PI3Kα, HDAC1/2/3/10)
TGX-221 (PI3Kβ specific)
Normalized Cell Viability (%)
Concentration (μM)
SNU-387
SNU-398
SNU-387 Empty Vector
SNU-387 Tg:SALL4A
SNU-387 Tg:SALL4B


A
Urea Cycle
EV
Tg:SALL4A
Tg:SALL4B
Fold Change
0.427
0.127
*0.0107
*0.0330
**1.18E-3
**2.72E-3
*0.0272
*0.0379
**6.45E-3
Carbamoyl phosphate
Ornithine
Citrulline
Aspartate
Arginino-succinate
Arginine
Fumarate
Urea
Student's t-test
*P < 0.05
**P < 0.01
***P < 0.001
B
Urea Cycle Genes
Input
H3K27Ac
SALL4
CPS1
OTC
ASS1
ASL
ARG1
ARG2
C
Mitochondrial DNA quantification
SNU-387
SNU-398
SNU-387 Empty Vector
SNU-387 Tg:SALL4A
SNU-387 Tg:SALL4B
Relative Fold Change (ΔΔC_T)
*0.0381
**6.36E-3
*0.0270
**5.65E-3
**2.52E-3
***2.65E-4
***3.67E-4
*0.0294
**2.34E-3
Minor Arc
ND1
ND4
D
Mitochondrial Biogenesis Genes
EV
Tg:SALL4A
Tg:SALL4B
Relative Fold Change (ΔΔC_T)
**7.35E-3
ACTB
PGC-1α
PGC-1β
TFAM
NRF1
NRF2
E
Mitochondrial Biogenesis Genes
Input
H3K27Ac
SALL4
MIR573
PGC1α
PGC1β
NRF1
MIR182
NRF2
HNRNPA3
TFAM