Online Methods

Cell lines. HUH7 and Hep3B cells were obtained from Japanese Collection of Research Biorescources (JCRB) and ATCC, respectively. RIL175 cells were kindly provided by Prof. Simon Rothenfußer (Center of Integrated Protein Science Munich (CIPS-M) and Division of Clinical Pharmacology, Department of Internal Medicine IV, Klinikum der Universität München). For the cultivation of HUH7 and RIL175 DMEM (PAN Biotech GmbH, Aidenbach, Germany) supplemented with 10% FCS (Biochrom AG, Berlin, Germany) was used, while Hep3B cells were cultured in MEM Eagle (PAN Biotech GmbH, Aidenbach, Germany) supplemented with 10% FCS. All cells were cultured at 37 °C with 5% CO2 in constant humidity in an incubator. Before cell seeding, all culture flasks, multiwell-plates and dishes were coated with collagen G (0.001% in PBS, Biochrom AG, Berlin, Germany).

Compounds. (R)-Roscovitine was obtained from Sigma-Aldrich. Sorafenib was obtained from Enzo Life Sciences. Dinaciclib and Gefitinib was obtained from Selleckchem. LGR1407 was kindly provided by Libor Havlíček (Institute of Experimental Botany AS CR, Prague, Czech Republic) (1).

Transfection experiments – Cdk5 shRNA. For the transduction of HUH7 and Hep3B cells with Cdk5 shRNA and nt shRNA, Cdk5 MISSION® shRNA Lentiviral Transduction Particles (Vector: pLKO.1-puro; SHCLNV-NM_004935; Clone ID: (1) TRCN0000021465, (2) TRCN0000021466, (3) TRCN0000021467, (4) TRCN0000194974, (5) TRCN0000195513; Sigma-Aldrich, Taufkirchen, Germany) and MISSION® pLKO.1-puro Non-Mammalian shRNA Control Transduction Particles (SHC002V; Sigma-Aldrich, Taufkirchen, Germany) as a non-targeting control were used according to the manufacturer's protocol. Both cell lines were transduced with a multiplicity of infection (MOI) of one. Successfully transduced cells were selected by adding 2 µg/ml puromycin (Thermo Fisher Scientific, Waltham, MA) to the medium. After the initial selection, puromycin concentration was reduced to 1 µg/ml for

28 further cultivation to ensure the stable transfection with Cdk5 and nt shRNA. By using
29 Western Blot analysis the most efficient and well tolerated clones were selected.
30
31 **Genome engineering using the CRISPR/Cas9 system.** For the knockout of Cdk5 in murine
32 RIL175 cells the CRISPR-Cas9 system was used as described previously (2). In short, we
33 decided to introduce an InDel-mutation in exon 2 of Cdk5. Therefore we sequenced the
34 genomic region of interest (Cdk5 sequencing primer: 5'-GAGTTTATGGCAGATTCTCC-3')
35 and designed single guide RNAs (sgRNAs) as described previously (3). The three top-
36 ranked sgRNAs were used for further experiments and were inserted into the
37 eCas9_Puro2.0 plasmid using the T4 DNA ligase protocol provided by the manufacturer
38 (New England BioLabs, Frankfurt a.M., Germany). After transformation into competent
39 DH5α-E.coli, plasmids were isolated using the QIAGEN Plasmid Maxiprep Kit (Qiagen,
40 Hilden, Germany) according to the manufacturer's protocol and sequenced starting from the
41 U6 promotor to validate the correct insertion. RIL175 wild-type cells were then transfected
42 with the respective plasmids using Lipofectamine™ 3000 reagent (Invitrogen, Hilden,
43 Germany) as described by the manufacturer. After selection with puromycin, genome
44 targeting efficiency was checked using T7 DNA endonuclease I as described by the
45 manufacturer (New England BioLabs, Frankfurt a.M., Germany). Cells transfected with the
46 most efficient sgRNA (Cdk5 sgRNA top: 5'-CACCGGCTCTGAAGCGTGTCAGGC-3'; bottom:
47 5'-AAACGCCTGACACGCTTCAGAGCC-3') were diluted for clonal selection. Gene knockout
48 in identified clones was confirmed with sequencing and Western blot. Sequencing services,
49 sequencing primers, cloning oligomers and PCR primers were provided by Eurofins
50 Genomics GmbH (Ebersberg, Germany).
51
52 **Proliferation assay.** The proliferation of HCC cells was evaluated using the xCELLigence
53 system (Roche Diagnostics, Mannheim, Germany). The respective cell lines were seeded at
54 the given density in 100 µl growth medium in equilibrated 16-well E-plates (HUH7: 2 x 10^3
55 cells per well; Hep3B: 4 x 10^3 cells per well). After an initial incubation of 24 h without any

compounds, cells were either treated with different substances for 72 h or left untreated as a control (4 wells per experimental condition). Through impedance measurement, the xCELLigence system evaluates the cell index, a dimensionless parameter, which is proportional to the cell number and recorded every hour. In each figure, the cell index is displayed as one respresentative experiment. After normalizing the cell index to the start point of treatment, the doubling time was evaluated by the xCELLigence software and served for statistical evaluation. Doubling time was calculated from at least three independent experiments.

For the evaluation of synergism, two separate methods were used. First, the Combination Subthresholding approach was used where synergy is assumed, if both single treatments alone show no significant benefit over the untreated control, while the combination of both treatments shows a significant effect (4). Secondly, synergism was calculated using the Bliss Independence model (5). Therefore, the Bliss Value (BV) was evaluated by comparing the effects of drug A (E_A) and drug B (E_B) with the effect of the combination of both drugs (E_{AB}) according to the following formula:

$$BV = \frac{E_{AB}}{(E_A + E_B) - (E_A \times E_B)}$$

Synergistic effects were assumed with BV>1, antagonistic effects with BV<1 and additive effects with BV=1.

Clonogenic assay. For the evaluation of long term cell survival, cells were seeded into 6-well plates and treated with the respective compounds for 24 h. After the incubation, cells were trypsinized and reseeded at a density of 1 x 10^4 cells per well into a 6-well plate. After incubation for 7 d, viable cells were stained with crystal violet solution for 10 min (RT). Bound dye was solubilized by adding 1 ml dissolving buffer and the absorbance at 550 nm was measured using a SpectraFluor Plus™ plate reader (Tecan, Crailsheim, Germany).

Immunohistochemistry. 5 µm sections of paraffin embedded tumors from the xenograft mouse model were used for IHC. Therefore the slides were first deparaffinized and rehydrated. Thereafter the sections were boiled in sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0) for antigen retrieval, before endogenous peroxidase was blocked by incubation in 7.5% hydrogen peroxide for 10 min. As an indicator for proliferating cells a primary antibody against Ki67 (1:100, Abcam, ab15580) was applied for 1 h at room temperature. The Vectastain® Universal Elite ABC Kit (Vector Laboratories, Burlingame, CA) was used for antibody detection according to the manufacturer's protocol and AEC (Thermo Fisher Scientific, Waltham, MA) was used as a chromogen. The slides were then counterstained with hematoxylin (Sigma Aldrich, Taufkirchen, Germany) for 1 min before being washed with distilled water. The sections were embedded in FluorSave™ Reagent mounting medium and covered with glass coverslips. Images were collected with an Olympus BX41 microscope and an Olympus DP25 camera (Olympus, Hamburg, Germany).

Cell cycle and apoptosis analysis. Cell cycle analysis and evaluation of apoptosis rates was performed as described by Nicoletti et. al. (6). In detail, cells were seeded at a densitiy of 8 x 10^4 cells per well into 24 well plates and treated with Sorafenib (5 µM, 24, 48 and 72 h). After incubation cells were trypsinized, washed with PBS and centrifuged (600 g, 4°C, 10 min). Further cells were permeabilized and stained by adding fluorochrome solution (FS) containing propidium iodide (Sigma Aldrich, Taufkirchen, Germany). After an overnight incubation at 4 °C, cells were analysed by flow cytometry on a FACSCalibur (Becton Dickinson, Heidelberg, Germany). For the determination of cell populations in different cell cycle phases and the percentage of apoptotic cells the FlowJo 7.6 analysis software (Tree Star Inc., Ashland, USA) was used.

Migration/invasion assays. To examine the migratory ability of HCC cells under the influence of various compounds, cells were first seeded into 6-well plates and either left untreated or pretreated with the indicated agent for 24 h. After pretreatment, cells were

trypsinized, centrifuged (1000 rpm, 5 min, RT) and resuspended in DMEM or DMEM containing chemotherapeutic agents. 1 x 10^5 cells per condition were seeded into collagen G coated Transwell® Permeable Supports (8 µm pore polycarbonate inserts, Corning Inc., New York, NY), which were then placed into a 24-well plate containing 700 µl DMEM (negative control) or DMEM containing 10% FCS per well. Cell were allowed to migrate for 16 h (HUH7) or 24 h (Hep3B) before being stained with crystal violet. Migrated cells were counted in 5 fields under a 10-fold objective lense of a Zeiss Axiovert 25 microscope (Zeiss, Jena, Germany) and imaged with a Canon 450D camera (Canon, Krefeld, Germany). Cell counting was performed by using the particle counter plugin of the ImageJ software. For the evaluation of invasive capabilities the Transwell® Permeable Supports were coated with Matrigel® (Corning Inc., New York, NY) to simulate extracellular matrix.

Proteomic analysis via LC-MS/MS.

Treatment of cells. Nt shRNA and Cdk5 shRNA HUH7 cells were seeded at a density of 0.35 x 10^6 cells per well into 6-well plates and treated with Sorafenib (0.5 µM, 5µM, 24h). After incubation cells were washed five times with PBS and detached with trypsin/EDTA. To remove excessive trypsin/EDTA cells were centrifuged (1000 rpm, 5 min, 4°C). Cell pellets were resuspended in 100 µl ice-cold PBS and stored at -80°C until further processing.

Sample processing. Per 1 x 10^5 cells 20 µl of 8 M urea / 0.4 M NH_4HCO_3 was added. Cells were lysed using an ultrasonic device (Sonoplus GM3200 with BR30 cup booster, Bandelin, Berlin, Germany) applying 10,000 kJ. For further homogenization, samples were centrifuged through QIA-Shredder devices (Qiagen, Hilden, Germany). Protein concentrations were determined by Bradford assays and adjusted to 0.6 mg/ml with 8 M urea/0.4 M NH_4HCO_3. To cleave bisulfide bonds, 25 µg of total protein was incubated with DTE at a concentration of 4.5 mM for 30 min and free sulfhydryl residues were blocked with iodoacetamide (final concentration 10 mM) for 30 min in the dark. After dilution with water to a concentration of 1 M urea, 0.5 µg porcine trypsin (Promega, Madison, WI, USA) was added and incubated overnight at 37 °C.

Liquid-chromatography mass spectrometry. Chromatography of peptides was performed on an EASY-nLC 1000 chromatography system (Thermo Scientific, Waltham, MA) coupled to an Orbitrap XL instrument (Thermo Scientific). 2.5 µg of peptides diluted in 0.1 % formic acid (FA) were transferred to a trap column (PepMap100 C18, 75 µm x 2 cm, 3 µm particles, Thermo Scientific) and separated at a flow rate of 200 nL/min (Column: PepMap RSLC C18, 75 µm x 50 cm, 2 µm particles, Thermo Scientific) using a 260 min linear gradients from 5 % to 25 % solvent B (0.1 % formic acid, 100 % ACN) and a consecutive 60 min linear gradient from 25 % to 50 % solvent B. For data acquisition, a top five data dependent CID method was used.

Proteomic data processing. For the quantitative analysis of the data obtained from the mass spectrometry screen the MaxQuant and Perseus software packages (provided by Max Planck Institute of Biochemistry, Munich) were used.

Quantitative real-time PCR analysis. For the isolation of mRNA from cell culture samples the Qiagen RNeasy Mini Kit (Qiagen, Hilden, Germany) was used according to the manufacturer's protocol. Concentration of mRNA in each sample was determined with the NanoDrop® ND-1000 spectrophotomer (Nanodrop Technologies, Erlangen, Germany). For the creation of cDNA templates from mRNA by reverse transcription the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) was used as described by the manufacturer. The Real-Time-Polymerase chain reaction was performed with the ABI 7300 Real Time PCR System (Applied Biosystems, Foster City, CA) using SYBR Green Master Mix (ThermoFisher Scientific, Waltham, MA) and respective primers. Actin was used as a housekeeping gene. In order to evaluate changes in mRNA levels the ΔΔCT method was used as described earlier (7).

Western blot analysis and antibodies. Proteins were separated on a SDS-PAGE gel, transferred to a nitrocellulose membrane (Hybond-ECL™, Amersham Bioscience) and incubated with a primary antibody overnight at 4 °C. The following antibodies were used:

pAkt (Ser473) (1:500, Cell Signaling Technologies, 9271), Akt (1:1000, Cell Signaling Technologies, 9272), pErk (Thr202/Tyr204) (1:1000, Cell Signaling Technologies, 9106), Erk (1:1000, Cell Signaling Technologies, 9102), pEGFR (Tyr1068) (1:1000, Cell Signaling Technologies, 2234), EGFR (1:1000, Cell Signaling Technologies, 2239), pH2A.X (1:1000, Cell Signaling Technologies, 2577), actin (1:1000, Merck Millipore, MAB1501), p62/Sequestosome1 (1:1000, Cell Signaling Technologies, 8025), LC3 (1:1000, Cell Signaling Technologies, 4108), FTH1 (1:1000, Sigma Aldrich, F5012), LIN28B (1:1000, Cell Signaling Technologies, 4196), CA2 (1:1000, ThermoFisher Scientific, PA5-28267), Vimentin (1:1000, Cell Signaling Technologies, 5741). As loading control, the stain-free technology (Bio Rad) was used. This technique enables a quantification of the whole lane protein, and therefore can be used for the normalization of protein bands (8). Proteins were visualized using horseradish peroxidase coupled secondary antibodies and ECL solution containing 2.5 mM luminol. Chemiluminescence was detected with the Chemidoc™ Touch Imaging system (Bio-Rad, Munich, Germany).

Glycolysis Stress Test. nt and Cdk5 shRNA HUH7 cells were seeded at a density of 1.5 x 10^4 into a XF^{e}96 microplate and grown for 24 h prior to Sorafenib treatment (0.5 µM, 5 µM, 24 h). The Seahorse Glycolysis Stress Test Kit was used in combination with the Seahorse XFe96 Analyzer (Agilent Technologies, Santa Clara, CA) as described by the manufacturer. Results were normalized to DNA content measured with CyQuant® GR dye solution (ThermoFisher Scientific, Waltham, MA) according to the manufactures protocol. Data analysis was performed with Wave 2.3.0 software and Seahorse XF Glycolysis Stress Test Report Generator (Agilent Technologies, Santa Clara, CA). For statistical analysis the following parameters were calculated from the respective graphs:

ECAR: Non-gylcolytic acidification (NGA), glycolysis (G), glycolytic capacity (GC) and glycolytic reserve (GR) (Table 2); OCR: Non-glycolytic OCR (NG-OCR) and glycolytic OCR (G-OCR) (Table 3).

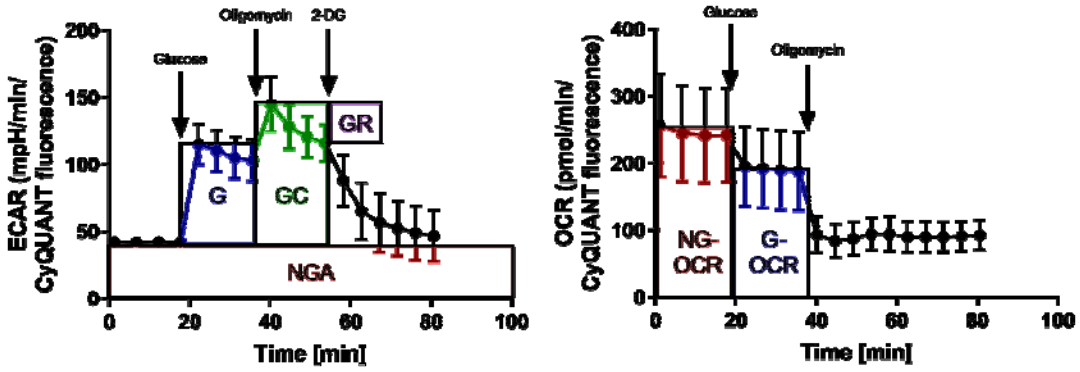

Figure 1 – Seahorse glycolysis stress test parameters for ECAR (left panel) and OCR (right panel).

Human HCC microarrays. Tissue microarrays (TMA), containing human HCC samples and matched surrounding non-tumor tissue were produced. The TMAs included 115 patients which had been treated with liver transplantation or partial hepatectomy at the University Clinic Munich Großhadern between 2008 and 2013. The formalin-fixed, paraffin-embedded blocks were cut into 2 mm thick slices and mounted on SuperFrost Plus microscope slides (Menzel Gläser, Braunschweig, Germany). After deparaffinization and rehydration all slides were Hematoxilien-Eosin stained in a standard manner (Vector Laboratories, Burlingame, CA, USA). Several blank-slides were set aside for immunohistochemical stainings.

Staining for EGFR was performed by using a Ventana Benchmark XT autostainer using the XT UltraView diaminobenzidine kit (Ventana Medical Systems). The Ventana EGFR-antibody clone 3C6 (ready to use) was used.

EGFR-staining of the TMA section was assessed using the immunoreactive score as described previously (9): 0 – absent; 1-4 –weak; 5-8 – moderate; 9-12 – strong expression. Images were obtained with a digital network microscope Leica DMD108 (Leica Biosystems Nussloch, Germany).

Statistical analysis. All listed experiments were conducted at least three times unless otherwise indicated in the figure legends. The given data is presented as mean ± SEM and

217 statistical significance was considered if $P \leq 0.05$. The statistical analysis was performed with
218 GraphPad Prism software version 7.0 (GraphPad Software, San Diego, USA).
219
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243

244 **Table 1 – PCR Primer**

Gene	forward primer	reverse primer
ABCB1	5'-TCGTAGGAGTGTCCGTGGAT-3'	5'-ATGTGCCACCAAGTAGGCTC-3'
AKR7A2	5'-TTCTACCTACACGCACCTGAC-3'	5'-ATAGTTGGAGAGGCCAAGCTC-3'
ANPEP	5'-ATCCCTGTCATCAATCGGGC-3'	5'-GTTGGGGTGGATCGGGTTAT-3'
APOB	5'-AATGGCCCCGTTTACCATGA-3'	5'-GGTCTTGAGTTTCCAGGTGC-3'
ARF5	5'-TGAGCGAGCTGACTGACAAG-3'	5'-GAAACCCAGATCCCTGCTCC-3'
CA2	5'-GTACGGCAAACACAACGGAC-3'	5'-CTGTAAGTGCCATCCAGGGG-3'
CARHSP1	5'-AGAACGGATTGCAGGGTCA-3'	5'-TCCCACAAGCACAGGACAAG-3'
CLDN1	5'-GCTGGGCTTCCCTAGATGTC-3'	5'-GAGGTGGGCAGTCCTTTGTT-3'
DDT	5'-GCCCTGACCCAGAAACGACT-3'	5'-CAGCTCCTCAGATGTCCGTG-3'
EGFR	5'-GGCCTAAGATCCCGTCCATC-3'	5'-TGGCTTTCGGAGATGTTGCT-3'
ENTPD5	5'-AAATTGCCTCTGCAGGTGTG-3'	5'-GCTGTCCTGGCATTTTCTGC-3'
FABP5	5'-AGGAGTGGGAATAGCTTTGCG-3'	5'-GCTGAACCAATGCACCATCT-3'
FAM115A	5'-GCGGGGCAAACAAAACCAATA-3'	5'-GGGAACGTGAACAGAACCCT-3'
FTH1	5'-TCAACAGTGCTTGGACGGAA-3'	5'-GTCCTGGTGGTAGTTCTGGC-3'
FTL	5'-GCTCCTTCTTGCCAACCAAC-3'	5'-GCCCAGAGAGAGGTAGGTGT-3'
G6PD	5'-TCCTGCATGAGCCAGATAGG-3'	5'-TGCGGTAGATCTGGTCCTCA-3'
GLDC	5'-AGAAACATCTCGCCCCGTTT-3'	5'-TCCGTCTTCCAACCATCAGC-3'
H2AFY	5'-GGCCCGGAAATCCAAGAAGA-3'	5'-ACACTTGTCTGCACCCCAAA-3'
HGD	5'-AGAGAGGAATGCGGTTCAGC-3'	5'-TTGCCTCATAGTGACCTCGG-3'
HPRT1	5'-AGGCGAACCTCTCGGCTTTC-3'	5'-AATCCGCCCAAAGGGAACTG-3'
HSD17B11	5'-TGCAAAGAAGGTGAAGGCAGAA-3'	5'-TGAAGACTGCCAAAGCCTCA-3'
HTATIP2	5'-CAGGGAAGGTGGGATGCTC-3'	5'-CCATTCACCTGGGCGAGATT-3'
IDI1	5'-CTTTCGGATTGGGAGGGCTT-3'	5'-GCCAATCACGCTTTCGATCC-3'
KPNA2	5'-TCGAGGTGGACCCTTTGAAC-3'	5'-TGGGGCACAACTCCTGTTTT-3'
LGAL3	5'-GCCAACGAGCGGAAAATGG-3'	5'-CAGGCCATCCTTGAGGGTTT-3'

LIN28B	5'-GGAAAGCACATTAGACCATGCG-3'	5'-TATCCAAGGGGCTTCCCTCT-3'
NCEH1	5'-CGAAGAGCCACTGAAACGCA-3'	5'-GACCAGGACGCACTTGCACT-3'
P4HA1	5'-CAGTACATGACCCTGAGACTGG-3'	5'-CGTGCAAAGTCAAAATGGGGT-3'
PSMD12	5'-GCTAGCCAAGGAAGGAAGACT-3'	5'-CATCCGTTGCAGGACTCTCA-3'
PYCR1	5'-CCCTCTCCCCGTACTTTTCC-3'	5'-CCCATCTTCACACCCCCATC-3'
RAB10	5'-ATTTTGTCCCGACCGACTCC-3'	5'-ACGAAAAAGGACGCAGGTCT-3'
RBX1	5'-GTACTGTCGCATGGGGAGTC-3'	5'-ACTCTGCCTTGAGCTGTTGG-3'
RCN1	5'-ATGTTTGTCGGAAGCCAAGC-3'	5'-TCACTGGACTGGATGGGACA-3'
S100P	5'-AGTTCATCGTGTTCGTGGCT-3'	5'-CACTTTTGGGAAGCCTGGGA-3'
SCARB1	5'-GTCCATCTACCCACCCAACG-3'	5'-CCCTACAGTTTTGCTTCCTGC-3'
SCFD1	5'-GAAAGGCAGACAGTGGCTTTG-3'	5'-TAAGGGCCTCTGGAAGCTGA-3'
SDCBP	5'-AACCCTGCCAATCCAGCAAT-3'	5'-GGTCTTGCTACCAACTGCCC-3'
SFN	5'-ACTACGAGATCGCCAACAGC-3'	5'-CAGTGTCAGGTTGTCTCGCA-3'
SQSTM1	5'-CTCCGCGTTCGCTACAAAAG-3'	5'-TCCTCGTCACTGGAAAAGGC-3'
SULT2A1	5'-AGTGAAACGGAGAGTCCACG-3'	5'-GGCATCCAGCCATGAATGTG-3'
TF	5'-GAGTATGCGAACTGCCACCT-3'	5'-GCTGTAGGGAAAGACCAGACG-3'
TOP2A	5'-GCGGGCTAAAGGAAGGTTCAA-3'	5'-ACTAAACAGGCAGGACCCCA-3'
TPD52L2	5'-AGTGACCCAGTCAGACCTCTA-3'	5'-AGCTGCTGAGGGTCAGTTTC-3'
VIM	5'-CGGCGGGACAGCAGG-3'	5'-TCGTTGGTTAGCTGGTCCAC-3'

Table 2 – ECAR Parameters

ECAR Parameter	Rate Measurement Equation	Abbreviation
Non-Glycolytic Acidification	Last rate measurement before glucose injection	NGA
Glycolysis	(Maximum rate measurement before Oligomycin injection) - (Last rate measurement before Glucose injection)	G
Glycolytic Capacity	(Maximum rate measurement after Oligomycin injection) - (Last rate measurement before Glucose injection)	GC
Glycolytic Reserve	(Glycolytic Capacity) - (Glycolysis)	GR

248 **Table 3 – OCR Parameters**

OCR Parameter	Rate Measurement Equation	Abbreviation
Non-Glycolytic OCR	Last rate measurement before glucose injection	NG-OCR
Glycolytic OCR	Minimum rate measurement before Oligomycin injection	G-OCR

249

Supplementary Fig. 1. Combination of Cdk5 inhibition and Sorafenib.
(A) Western Blot showing Cdk5 protein levels in non-targeting (nt) and Cdk5 shRNA HUH7 cells. (B) Western Blot showing Cdk5 protein levels in non-targeting (nt) and Cdk5 shRNA Hep3B cells (C) Western Blot showing Cdk5 protein levels in RIL175 wild-type cells and RIL175 Cdk5 knockout (Cdk5 KO) cells generated via the CRISPR-Cas9 method. (D) Clonogenic survival of HUH7 cells treated with either Sorafenib (5 µM), roscovitine (10 µM) or combination of both is shown. One Way ANOVA, Tukey $^{**}P < 0.01$, n=3. (E) Clonogenic survival of HUH7 cells treated with Sorafenib is shown. t-test $^{*}P < 0.05$, n=3.

Supplementary Fig. 2. Combination of Cdk5 inhibition and Sorafenib does not affect DNA damage, cell cycle and apoptosis
(A) Immunoblot of non-targeting (nt) and Cdk5-1/4 shRNA HUH7 cells treated with Sorafenib and probed for phosphorylated H2A.X is shown. (B) Apoptosis of Sorafenib treated non-targeting (nt) and Cdk5 shRNA HUH7 cells is shown. One Way ANOVA, Tukey *P<0.05, n=3. (C) Cell cycle analysis of non-targeting (nt) and Cdk5 shRNA HUH7 cells after treatment with Sorafenib is shown. The bar graph displays respective quantitative evaluations. ns: not significant; unpaired t-test, n=3

Supplementary Fig. 3. Proteomic analysis of Cdk5 knockdown cells treated with Sorafenib
(A) Table of proteins showing alterations of protein abundance ($P < 0.05$; log2-fold change > |0.6|) between non-targeting (nt) and Cdk5 shRNA HUH7 cells treated with Sorafenib together with their respective gene names, x-fold changes (nt 5 µM Sorafenib vs. Cdk5 5 µM Sorafenib) and *P*-values. (B) Volcano Plot visualizing the protein hits given in *A* is shown. (C) Protein interaction map of protein hits given in *A* created with string-db.org. (protein-protein interaction enrichment *P*-value: $1.57*10^{-6}$).

Supplementary Fig. 4. Analysis of targets from the proteomic screen
Bar graphs display mRNA expression of selected proteins yielded from the proteomic analysis of untreated (A) or Sorafenib treated (B) nt and Cdk5 shRNA HUH7 cells. Paired t-test, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, n=3. (C) Representative immunoblots of nt and Cdk5 shRNA HUH7 cells treated with Sorafenib and probed with antibodies against proteins of interest (Carbonic Anhydrase 2 (CA2), Lin28B, Ferritin Heavy Chain (FTH), Vimentin) from the proteomics screen are shown.

Supplementary Fig. 5. Sorafenib affects the metabolism of HCC cells
Glycolysis Stress Test of non-targeting (nt) and Cdk5 shRNA HUH7 cells that were pre-treated with Sorafenib before consecutive exposure to D-glucose, oligomycin and 2-DG is shown. ECAR and OCR were recorded using a Seahorse XFe96 Analyzer and normalized with CyQUANT® GR dye. (A-E) Normalized ECAR (upper left) and OCR (upper right) of untreated and Sorafenib treated non-targeting (nt) and Cdk5 shRNA HUH7 cells are compared. For statistical analysis the following parameters were compared for ECAR (lower left): NGA (non-glycolytic acidification), G (glycolysis), GC (glycolytic capacity), GR (glycolytic reserve) and for OCR (lower right): NG-OCR (non-glycolytic oxygen consumption rate), G-OCR (glycolytic oxygen consumption rate). Multiple t-tests, ns: not significant n=3.

Supplementary Fig. 6. Cdk5 inhibition prevents compensatory EGFR activation in Hep3B cells after Sorafenib treatment.

Immunoblots from non-targeting (nt) and Cdk5 shRNA Hep3B cells treated with Sorafenib probed with antibodies for phosphorylated EGFR (p-EGFR), EGFR (A), phosphorylated Akt (p-Akt) and Akt (B), phosphorylated Erk (p-Erk), Erk (C), are shown. n=2.

Supplementary Fig. 7. EGFR protein levels are high in human HCC.
(A) Immunostaining of patient tissue for EGFR (red) in HCC tissue and healthy liver tissue is shown. (B) Percentage of EGFR positive cells in HCC tissue is shown. (C) Staining intensity of EGFR in HCC tissue is shown. (D) Immunoreactive score for EGFR in HCC tissue is shown.

Supplementary Table 1. Correlation of EGFR staining with clinical parameters
Contingency tables correlating percentage of EGFR positive cells, EGFR staining intensity and EGFR IRS with r-classification (R0: no residual tumor, R1: residual tumor, X: N/A) (A), frequency of recurrence (0: no tumor recurrence, 1: tumor recurrence) (B), cause of death (C), tumor stage (D) and tumor grading (E) are shown.

Supplementary Video 1.
Live cell imaging of nt shRNA (left) and Cdk5 shRNA (right) HUH7 cells expressing eGFP-EGFR is shown.

Supplementary Video 2.
Live cell imaging of nt shRNA (left) and Cdk5 shRNA (right) Hep3B cells expressing eGFP-EGFR is shown.

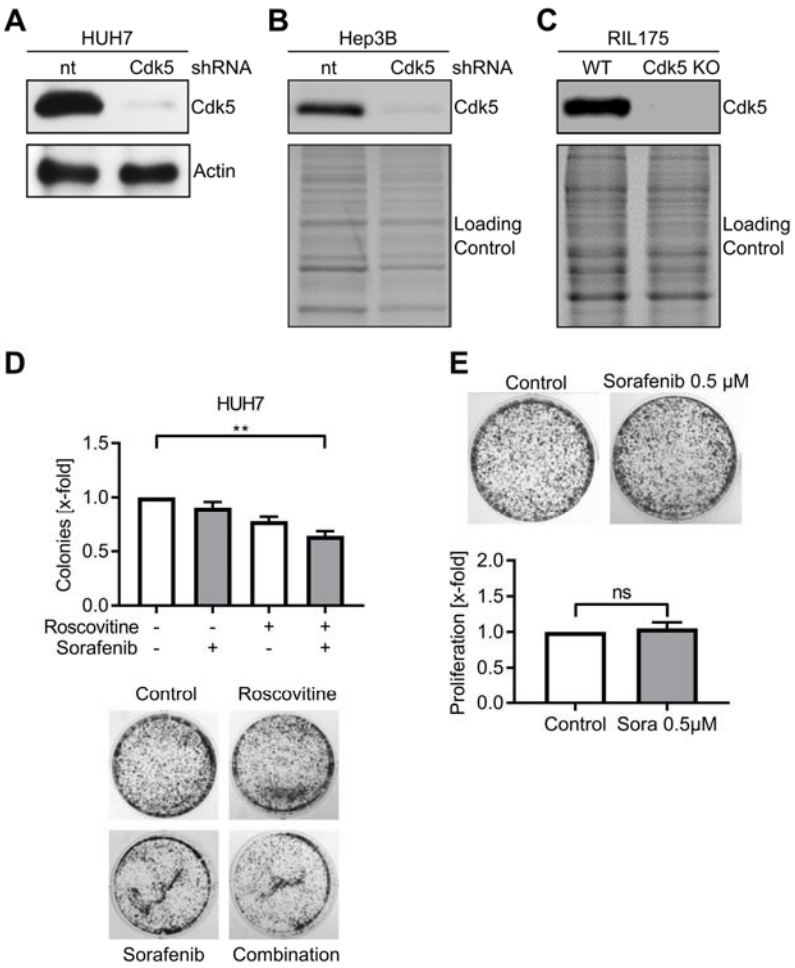
Supplementary Video 3.
Live cell imaging of nt shRNA (left) and Cdk5 shRNA (right) HUH7 cells expressing eGFP-Integrin α5 (ITGA5) is shown.

Supplementary Video 4.
Live cell imaging of nt shRNA (left) and Cdk5 shRNA (right) Hep3B cells expressing eGFP-Integrin α5 (ITGA5) is shown.

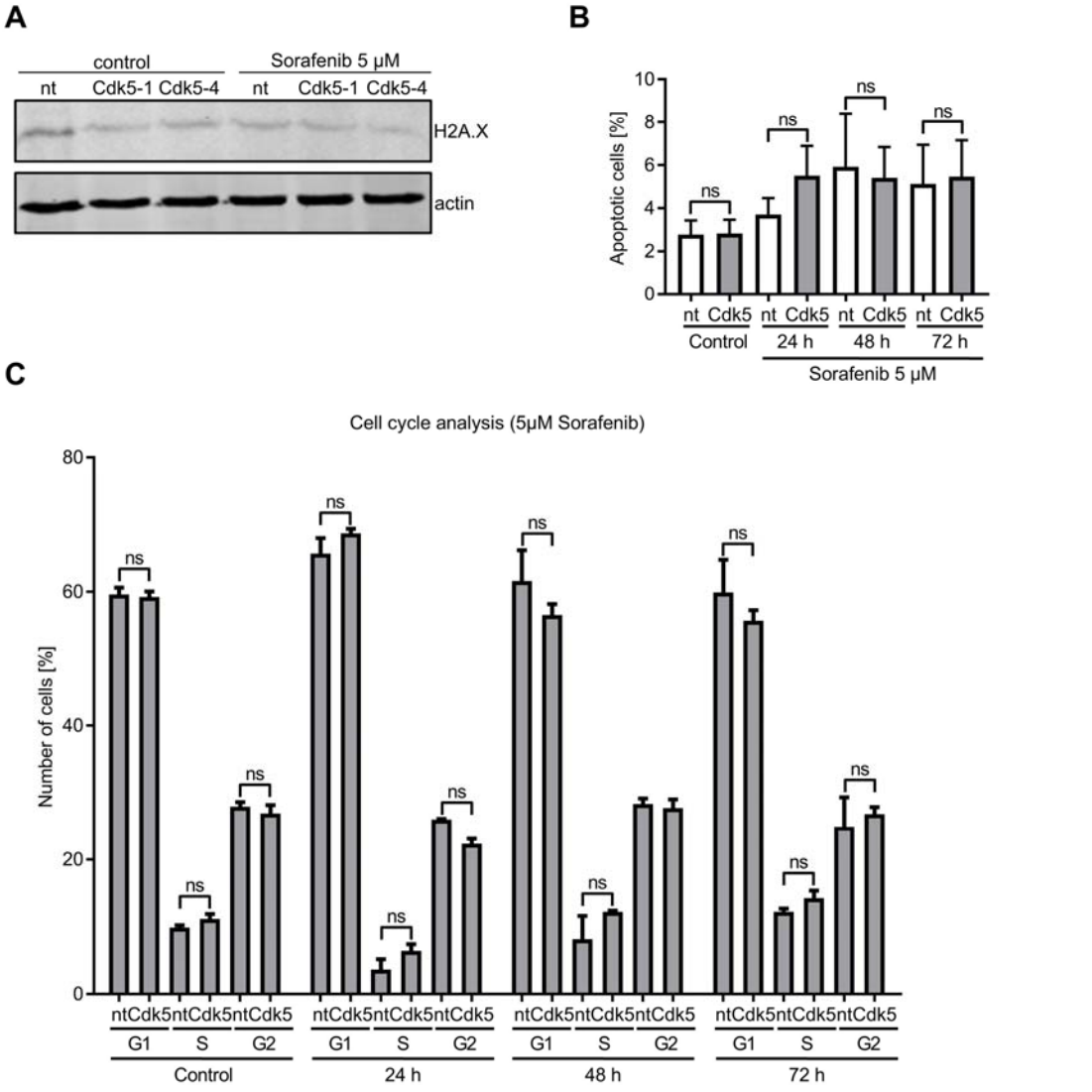
Supplementary Video 5.
Live cell imaging of nt shRNA (left) and Cdk5 shRNA (right) HUH7 cells expressing eGFP-c-Met is shown.

Supplementary Video 6.
Live cell imaging of nt shRNA (left) and Cdk5 shRNA (right) Hep3B cells expressing eGFP-c-Met is shown.

Supp. Fig. 1


A
HUH7
nt
Cdk5
shRNA
Cdk5
Actin
B
Hep3B
nt
Cdk5
shRNA
Cdk5
Loading Control
C
RIL175
WT
Cdk5 KO
Cdk5
Loading Control
D
HUH7
**
Colonies [x-fold]
1.5
1.0
0.5
0.0
Roscovitine - - + +
Sorafenib - + - +
Control
Roscovitine
Sorafenib
Combination
E
Control
Sorafenib 0.5 µM
Proliferation [x-fold]
2.0
1.5
1.0
0.5
0.0
ns
Control
Sora 0.5µM

Supp. Fig. 2

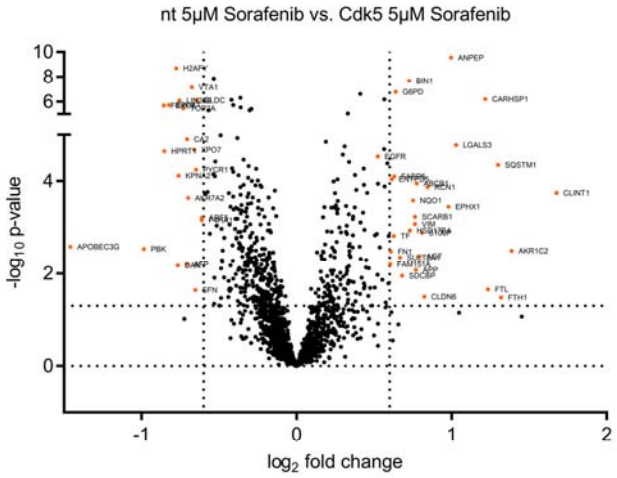

A
control
Sorafenib 5 µM
nt
Cdk5-1
Cdk5-4
nt
Cdk5-1
Cdk5-4
H2A.X
actin
B
Apoptotic cells [%]
ns
nt
Cdk5
Control
24 h
48 h
72 h
Sorafenib 5 µM
C
Cell cycle analysis (5µM Sorafenib)
Number of cells [%]
G1
S
G2
Control
24 h
48 h
72 h

Supp. Fig. 3

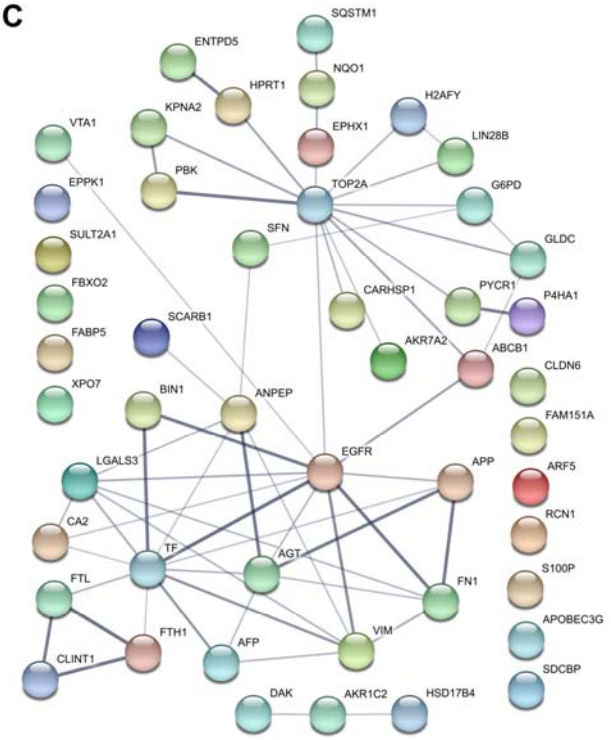
A

Protein names	Gene names	x-fold change	t-test p-value
DNA dC->dU-editing enzyme APOBEC-3G	APOBEC3G	0.36	0.00263
Lymphokine-activated killer T-cell-originated protein kinase	PBK	0.51	0.00295
F-box only protein 2	FBXO2	0.55	2.01E-06
Hypoxanthine-guanine phosphoribosyltransferase	HPRT1	0.55	2.26E-05
Epiplakin	EPPK1	0.57	1.91E-06
Core histone macro-H2A.1;Histone H2A	H2AFY	0.58	1.97E-09
Bifunctional ATP-dependent dihydroxyacetone kinase/FAD-AMP lyase (cyclizing)	DAK	0.59	0.00665
Importin subunit alpha	KPNA2	0.59	7.62E-05
Protein lin-28 homolog B	LIN28B	0.59	8.00E-07
DNA topoisomerase 2-alpha	TOP2A	0.60	3.41E-06
Alpha-fetoprotein	AFP	0.61	0.00639
Carbonic anhydrase 2	CA2	0.61	1.24E-05
Aflatoxin B1 aldehyde reductase member 2	AKR7A2	0.62	0.00023
Vacuolar protein sorting-associated protein VTA1 homolog	VTA1	0.63	7.07E-08
Exportin-7	XPO7	0.63	2.11E-05
14-3-3 protein sigma	SFN	0.64	0.02276
Pyrroline-5-carboxylate reductase 1. mitochondrial	PYCR1	0.64	5.71E-05
Glycine dehydrogenase [decarboxylating]. mitochondrial	GLDC	0.64	7.68E-07
Prolyl 4-hydroxylase subunit alpha-1	P4HA1	0.65	0.00070
ADP-ribosylation factor 5	ARF5	0.65	0.00062
Epidermal growth factor receptor	EGFR	1.44	2.95E-05
Protein FAM151A	FAM151A	1.52	0.00627
Fibronectin	FN1	1.52	0.00335
Ectonucleoside triphosphate diphosphohydrolase 5	ENTPD5	1.53	9.08E-05
Serotransferrin	TF	1.54	0.00154
Fatty acid-binding protein. epidermal	FABP5	1.55	7.90E-05
Glucose-6-phosphate 1-dehydrogenase	G6PD	1.56	1.59E-07
Bile salt sulfotransferase	SULT2A1	1.59	0.00454
Syntenin-1	SDCBP	1.60	0.01119
Myc box-dependent-interacting protein 1	BIN1	1.65	2.07E-08
Peroxisomal multifunctional enzyme type 2	HSD17B4	1.66	0.00117
NAD(P)H dehydrogenase [quinone] 1	NQO1	1.68	0.00026
Vimentin	VIM	1.70	0.00085
Scavenger receptor class B member 1	SCARB1	1.70	0.00059
Amyloid beta A4 protein	APP	1.71	0.00840
Multidrug resistance protein 1	ABCB1	1.71	0.00011
Angiotensinogen	AGT	1.73	0.00424
Protein S100-P	S100P	1.75	0.00131
Claudin-6	CLDN6	1.77	0.03187
Reticulocalbin-1	RCN1	1.80	0.00014
Epoxide hydrolase 1	EPHX1	1.97	0.00036
Aminopeptidase N	ANPEP	1.99	2.82E-10
Galectin-3	LGALS3	2.04	1.67E-05
Calcium-regulated heat stable protein 1	CARHSP1	2.33	6.35E-07
Ferritin;Ferritin light chain	FTL	2.35	0.02201
Sequestosome-1	SQSTM1	2.46	4.46E-05
Ferritin;Ferritin heavy chain	FTH1	2.49	0.03318
Aldo-keto reductase family 1 member C2	AKR1C2	2.62	0.00323
Clathrin interactor 1	CLINT1	3.20	0.00018

B

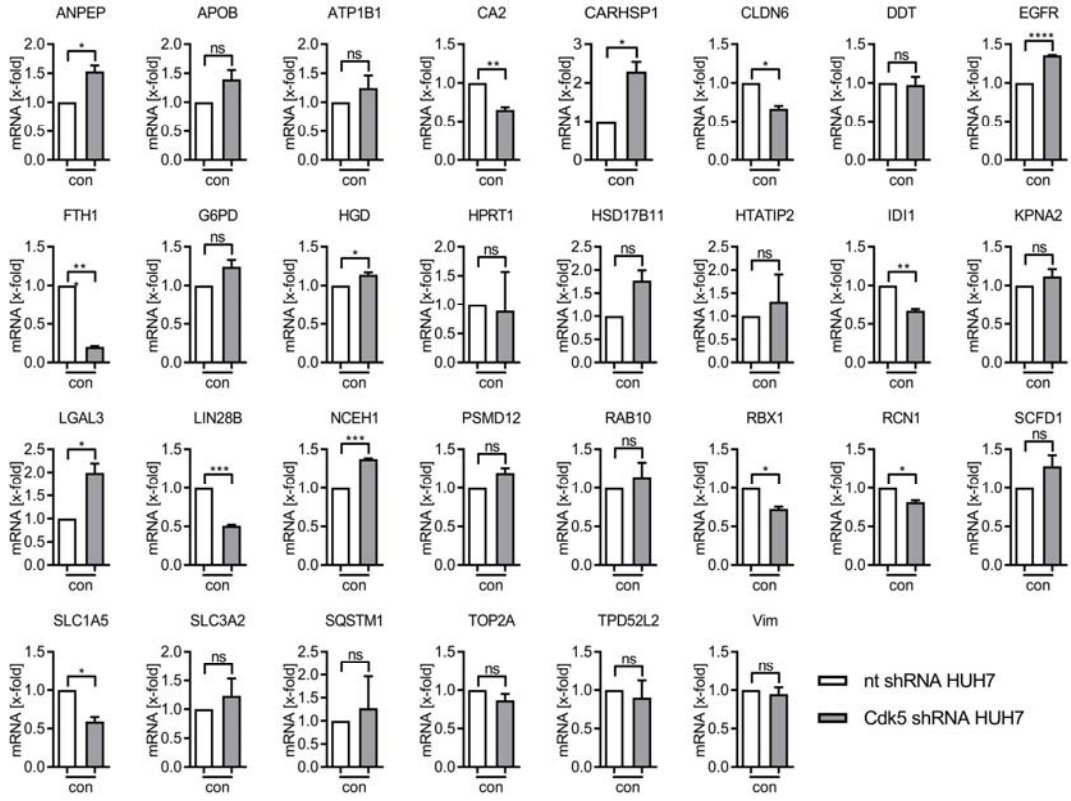


C

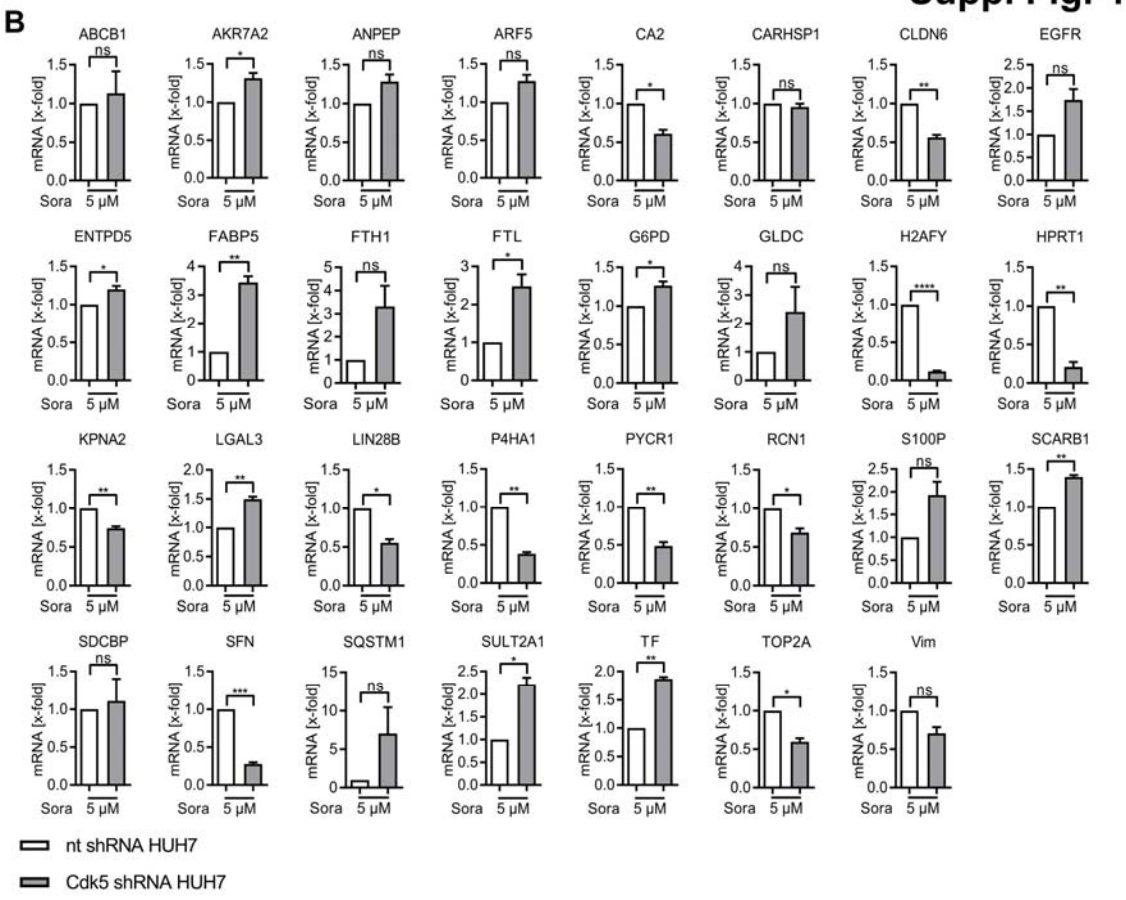


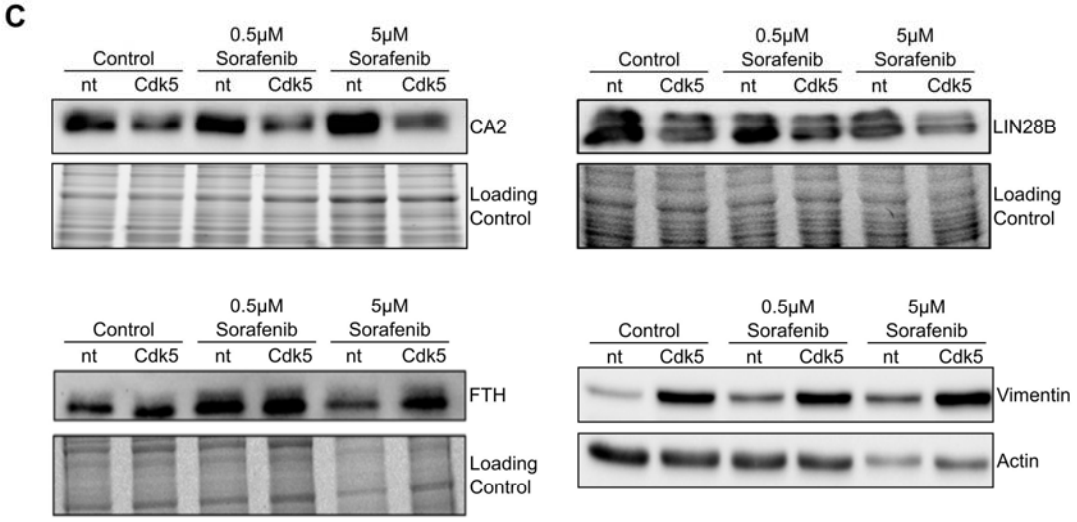
A

Supp. Fig. 4

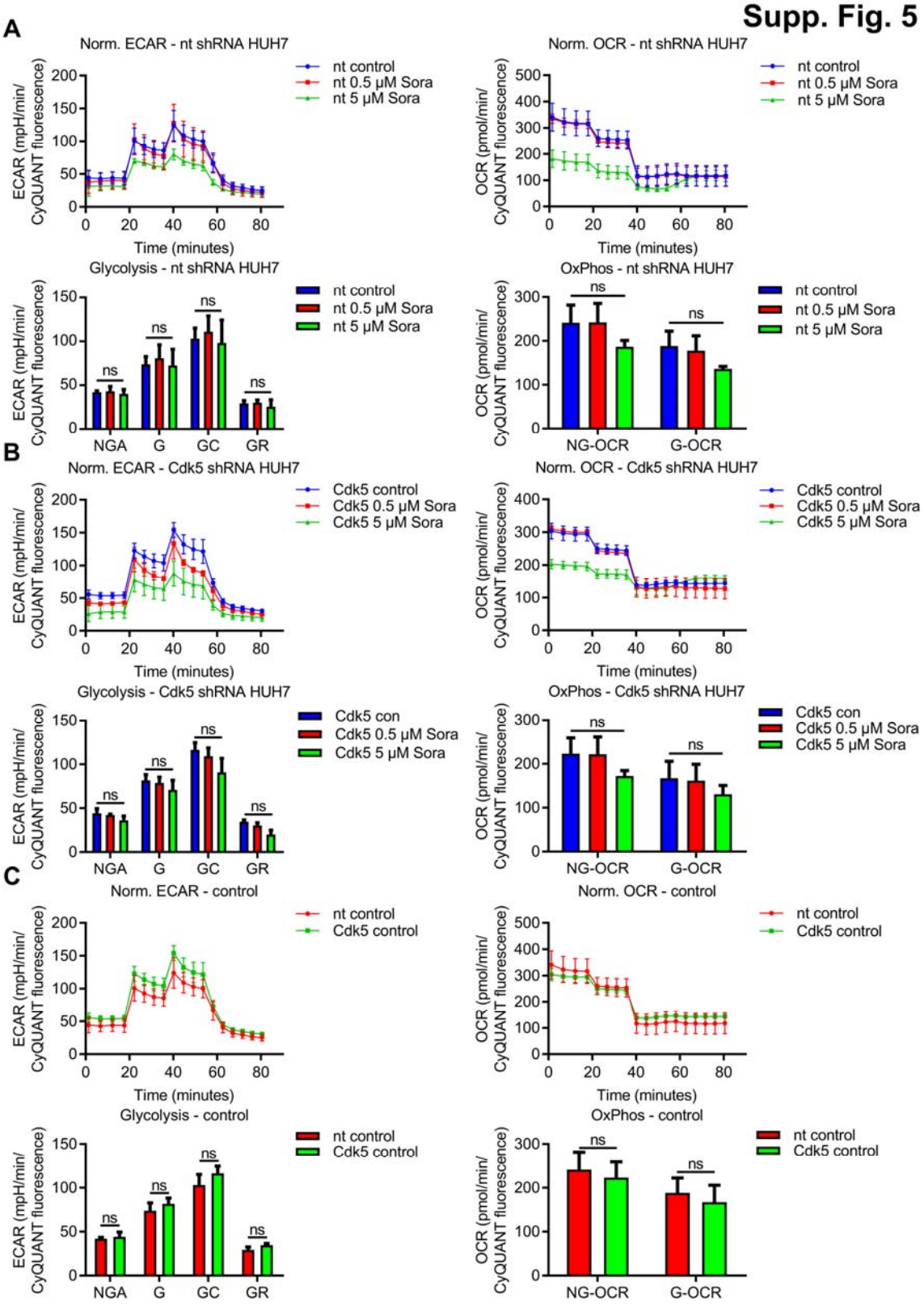

ANPEP
APOB
ATP1B1
CA2
CARHSP1
CLDN6
DDT
EGFR
FTH1
G6PD
HGD
HPRT1
HSD17B11
HTATIP2
IDI1
KPNA2
LGAL3
LIN28B
NCEH1
PSMD12
RAB10
RBX1
RCN1
SCFD1
SLC1A5
SLC3A2
SQSTM1
TOP2A
TPD52L2
Vim
mRNA [x-fold]
con
nt shRNA HUH7
Cdk5 shRNA HUH7

Supp. Fig. 4

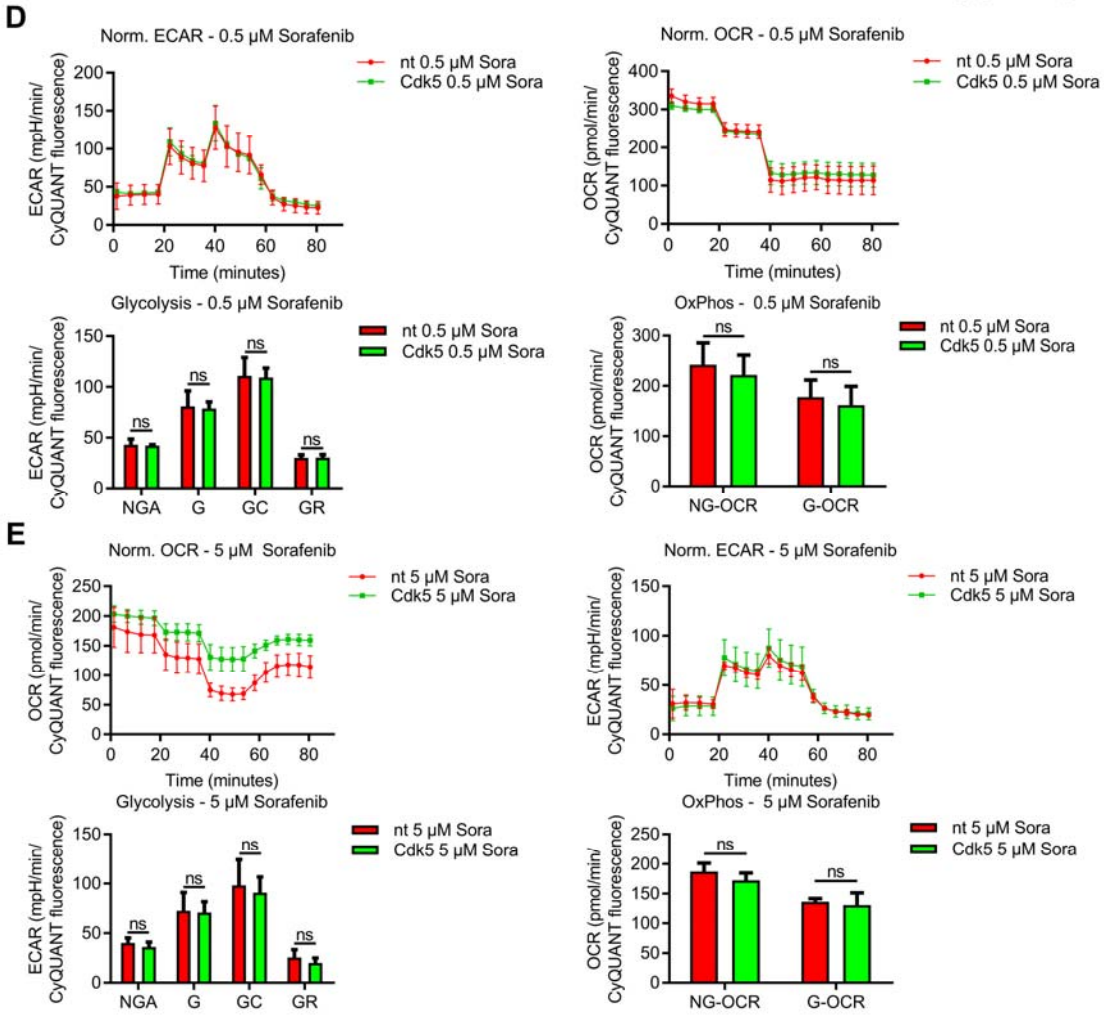

B
ABCB1
AKR7A2
ANPEP
ARF5
CA2
CARHSP1
CLDN6
EGFR
ENTPD5
FABP5
FTH1
FTL
G6PD
GLDC
H2AFY
HPRT1
KPNA2
LGAL3
LIN28B
P4HA1
PYCR1
RCN1
S100P
SCARB1
SDCBP
SFN
SQSTM1
SULT2A1
TF
TOP2A
Vim
mRNA [x-fold]
Sora
5 µM
ns
nt shRNA HUH7
Cdk5 shRNA HUH7


C
Control
0.5µM Sorafenib
5µM Sorafenib
nt
Cdk5
CA2
LIN28B
FTH
Vimentin
Loading Control
Actin

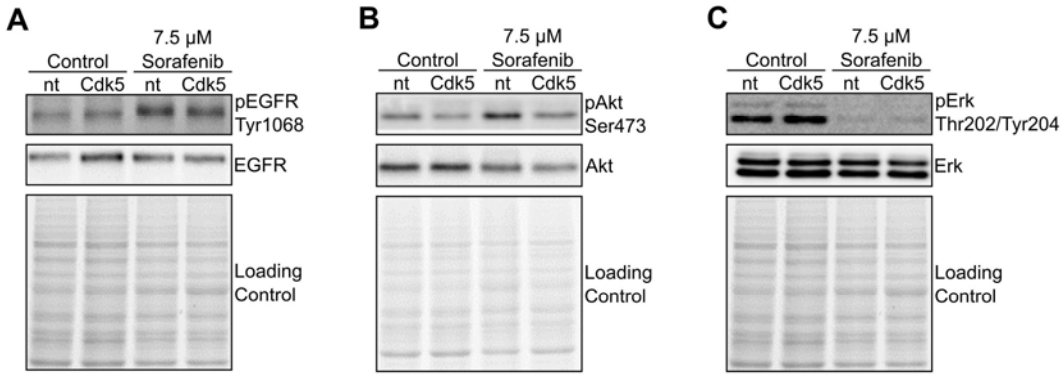
Supp. Fig. 5


A
Norm. ECAR - nt shRNA HUH7
ECAR (mpH/min/ CyQUANT fluorescence)
Time (minutes)
nt control
nt 0.5 µM Sora
nt 5 µM Sora
Norm. OCR - nt shRNA HUH7
OCR (pmol/min/ CyQUANT fluorescence)
Glycolysis - nt shRNA HUH7
NGA
G
GC
GR
ns
OxPhos - nt shRNA HUH7
NG-OCR
G-OCR
B
Norm. ECAR - Cdk5 shRNA HUH7
Cdk5 control
Cdk5 0.5 µM Sora
Cdk5 5 µM Sora
Norm. OCR - Cdk5 shRNA HUH7
Glycolysis - Cdk5 shRNA HUH7
Cdk5 con
OxPhos - Cdk5 shRNA HUH7
C
Norm. ECAR - control
Norm. OCR - control
Glycolysis - control
OxPhos - control

Supp. Fig. 5

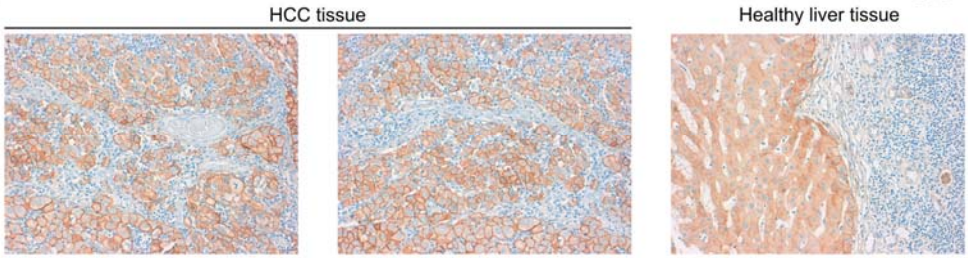

D
Norm. ECAR - 0.5 µM Sorafenib
nt 0.5 µM Sora
Cdk5 0.5 µM Sora
ECAR (mpH/min/ CyQUANT fluorescence)
Time (minutes)
Norm. OCR - 0.5 µM Sorafenib
OCR (pmol/min/ CyQUANT fluorescence)
Glycolysis - 0.5 µM Sorafenib
NGA
G
GC
GR
ns
OxPhos - 0.5 µM Sorafenib
NG-OCR
G-OCR
E
Norm. OCR - 5 µM Sorafenib
nt 5 µM Sora
Cdk5 5 µM Sora
Norm. ECAR - 5 µM Sorafenib
Glycolysis - 5 µM Sorafenib
OxPhos - 5 µM Sorafenib

Supp. Fig. 6


A
7.5 μM
Control
Sorafenib
nt
Cdk5
nt
Cdk5
pEGFR
Tyr1068
EGFR
Loading
Control
B
7.5 μM
Control
Sorafenib
nt
Cdk5
nt
Cdk5
pAkt
Ser473
Akt
Loading
Control
C
7.5 μM
Control
Sorafenib
nt
Cdk5
nt
Cdk5
pErk
Thr202/Tyr204
Erk
Loading
Control

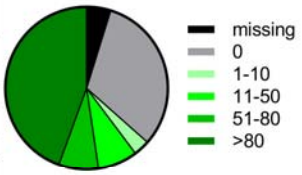
Supp. Fig. 7

A



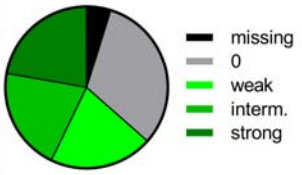
B

EGFR percentage					
		frequency	percentage	valid percentage	cumulated percentage
valid	negative	20	31.7	31.7	31.7
	1-10%	2	3.2	3.2	34.9
	11-50%	5	7.9	7.9	42.9
	51-80%	5	7.9	7.9	50.8
	>80%	28	44.4	44.4	95.2
	missing	3	4.8	4.8	100
	total	63	100	100	



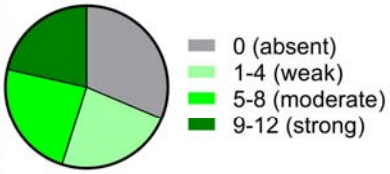
C

EGFR intensity					
		frequency	percentage	valid percentage	cumulated percentage
valid	0	20	31.7	31.7	31.7
	weak	13	20.6	20.6	52.4
	interm.	13	20.6	20.6	73
	strong	14	22.2	22.2	95.2
	missing	3	4.8	4.8	100
	total	63	100	100	



D

EGFR – IRS					
		frequency	percentage	valid percentage	cumulated percentage
valid	0	20	31.7	31.7	31.7
	1	1	1.6	1.6	33.3
	2	5	7.9	7.9	41.3
	4	9	14.3	14.3	55.6
	5	3	4.8	4.8	60.3
	6	3	4.8	4.8	65.1
	8	9	14.3	14.3	79.4
	9	2	3.2	3.2	82.5
	12	11	17.5	17.5	100
	total	63	100	100	



A

Supp. Table 1

R-classification					
		frequency	percentage	valid percentage	cumulated percentage
valid	R0	54	85.7	85.7	85.7
	R1	5	7.9	7.9	93.7
	X	4	6.3	6.3	100
	total	63	100	100	

R-classification * EGFR percentage of positive cells - contingency table								
number								
		EGFR percentage of positive cells						total
		negative	< 10%	11-50%	51-80%	> 80%	missing	
R-classification	R0	17	2	4	5	23	3	54
	R1	2	0	0	0	3	0	5
	X	1	0	1	0	2	0	4
total		20	2	5	5	28	3	63

chi-squared test			
	value	df	Asymptotic significance (two-sided)
Chi-squared (Pearson)	4.058[a]	10	0.945
Likelihood-Quotient	5.302	10	0.87
Number of valid cases	63		
a. 16 cells (88.9%) have an expected frequency of 5 or less. The smallest expected frequency is 0.13.			

R-classification * EGFR staining intensity – contingency table							
number							
		EGFR staining intensity					total
		0	weak	intermediate	strong	missing	
R-classification	R0	17	11	13	10	3	54
	R1	2	1	0	2	0	5
	X	1	1	0	2	0	4
total		20	13	13	14	3	63

chi-squared test			
	value	df	Asymptotic significance (two-sided)
Chi-squared (Pearson)	5.306[a]	8	0.724
Likelihood-Quotient	7.081	8	0.528
Number of valid cases	63		
a. 11 cells (88.9%) have an expected frequency of 5 or less. The smallest expected frequency is 0.19.			

100

Supp. Table 1

r-classification * EGFR IRS – contingency table											
number											
		EGFR – IRS									total
		0	1	2	4	5	6	8	9	12	
R-calssification	R0	17	1	4	8	3	3	9	2	7	54
	R1	2	0	0	1	0	0	0	0	2	5
	X	1	0	1	0	0	0	0	0	2	4
total		20	1	5	9	3	3	9	2	11	63

chi-squared test			
	value	df	Asymptotic significance (two-sided)
Chi-squared (Pearson)	10.252[a]	16	0.853
Likelihood-Quotient	12.064	16	0.74
Number of valid cases	63		
a. 23 cells (85.2%) have an expected frequency of 5 or less. The smallest expected frequency is 0.06.			

101

B

Supp. Table 1

Frequency of recurrence					
		frequency	percentage	valid percentage	cumulated percentage
valid	0	40	63.5	63.5	63.5
	1	17	27	27	90.5
	n.s.	6	9.5	9.5	100
	total	63	100	100	

Frequency of recurrance * EGFR percentage of positive cells - contingency table								
number								
		EGFR percentage of positive cells						total
		negative	< 10%	11-50%	51-80%	> 80%	missing	
frequency of recurrance	0	12	2	5	4	16	1	40
	1	7	0	0	1	8	1	17
	n.s.	1	0	0	0	4	1	6
total		20	2	5	5	28	3	63

chi-squared test			
	value	df	Asymptotic significance (two-sided)
Chi-squared (Pearson)	8.881[a]	10	0.543
Likelihood-Quotient	11.031	10	0.355
Number of valid cases	63		
a. 14 cells (77.8%) have an expected frequency of 5 or less. The smallest expected frequency is 0.19.			

Frequency of recurrence * EGFR staining intensity – contingency table							
number							
		EGFR staining intensity					total
		0	weak	intermediate	strong	missing	
frequency of recurrence	0	12	9	11	7	1	40
	1	7	4	1	4	1	17
	n.s.	1	0	1	3	1	6
total		20	13	13	14	3	63

chi-squared test			
	value	df	Asymptotic significance (two-sided)
Chi-squared (Pearson)	9.829[a]	8	0.277
Likelihood-Quotient	10.602	8	0.225
Number of valid cases	63		
a. 10 cells (66.7%) have an expected frequency of 5 or less. The smallest expected frequency is 0.29.			

102

Supp. Table 1

Frequency of recurrence * EGFR IRS – contingency table											
number											
		EGFR – IRS									total
		0	1	2	4	5	6	8	9	12	
frequency of recurrance	0	12	1	5	5	1	2	8	2	4	40
	1	7	0	0	4	1	1	0	0	4	17
	n.s.	1	0	0	0	1	0	1	0	3	6
total		20	1	5	9	3	3	9	2	11	63

chi-squared test			
	value	df	Asymptotic significance (two-sided)
Chi-squared (Pearson)	18.742[a]	16	0.282
Likelihood-Quotient	23.109	16	0.111
Number of valid cases	63		
a. 22 cells (81.5%) have an expected frequency of 5 or less. The smallest expected frequency is 0.10.			

103

C

Supp. Table 1

cause of death					
		frequency	percentage	valid percentage	cumulated percentage
valid	not determined	42	66.7	66.7	66.7
	tumor unrelated	2	3.2	3.2	69.8
	tumor related	14	22.2	22.2	92.1
	n.s.	5	7.9	7.9	100
	total	63	100	100	

cause of death * EGFR percentage of positive cells - contingency table								
number								
		EGFR percentage of positive cells						total
		negative	<10%	11-50%	51-80%	> 80%	missing	
cause of death	not determined	11	1	4	5	19	2	42
	tumor unrelated	1	0	0	0	1	0	2
	tumor related	7	1	1	0	4	1	14
	n.s.	1	0	0	0	4	0	5
total		20	2	5	5	28	3	63

chi-squared test			
	value	df	Asymptotic significance (two-sided)
Chi-squared (Pearson)	9.241[a]	15	0.865
Likelihood-Quotient	11.348	15	0.728
Number of valid cases	63		
a. 21 cells (87.5%) have an expected frequency of 5 or less. The smallest expected frequency is 0.06.			

cause of death * EGFR staining intensity – contingency table							
number							
		EGFR staining intensity					total
		0	weak	intermediate	strong	missing	
cause of death	not determined	11	11	10	8	2	42
	tumor unrelated	1	0	0	1	0	2
	tumor related	7	2	2	2	1	14
	n.s.	1	0	1	3	0	5
total		20	13	13	14	3	63

chi-squared test			
	value	df	Asymptotic significance (two-sided)
Chi-squared (Pearson)	10.536[a]	12	0.569
Likelihood-Quotient	11.373	12	0.497
Number of valid cases	63		
a. 16 cells (80.0%) have an expected frequency of 5 or less. The smallest expected frequency is 0.1.			

104

Supp. Table 1

cause of death * EGFR IRS – contingency table											
number											
		EGFR – IRS									total
		0	1	2	4	5	6	8	9	12	
cause of death	not determined	11	1	3	8	2	3	7	2	5	42
	tumor unrelated	1	0	0	0	0	0	0	0	1	2
	tumor related	7	0	2	1	1	0	1	0	2	14
	n.s.	1	0	0	0	0	0	1	0	3	5
total		20	1	5	9	3	3	9	2	11	63

chi-squared test			
	value	df	Asymptotic significance (two-sided)
Chi-squared (Pearson)	17.557[a]	24	0.824
Likelihood-Quotient	19.047	24	0.749
Number of valid cases	63		
a. 32 cells (88.9%) have an expected frequency of 5 or less. The smallest expected frequency is 0.03.			

105

D

Supp. Table 1

tumor score					
		frequency	percentage	valid percentage	cumulated percentage
valid	1	27	42.9	42.9	42.9
	2	17	27.0	27.0	69.8
	2a	2	3.2	3.2	73.0
	2b	2	3.2	3.2	76.2
	3	1	1.6	1.6	77.8
	3a	6	9.5	9.5	87.3
	3b	1	1.6	1.6	88.9
	4	1	1.6	1.6	90.5
	X	6	9.5	9.5	100.0
	total	63	100	100.0	

tumor score * EGFR percentage of positive cells - contingency table								
number								
		EGFR percentage of positive cells						total
		negative	< 10%	11-50%	51-80%	> 80%	missing	
valid	1	10	1	1	2	12	1	27
	2	5	1	3	1	6	1	17
	2a	0	0	0	1	1	0	2
	2b	1	0	0	1	0	0	2
	3	0	0	0	0	0	1	1
	3a	1	0	0	0	5	0	6
	3b	1	0	0	0	0	0	1
	4	0	0	0	0	1	0	1
	X	2	0	1	0	3	0	6
	total	20	2	5	5	28	3	63

chi-squared test			
	value	df	Asymptotic significance (two-sided)
Chi-squared (Pearson)	44.017[a]	40	0.305
Likelihood-Quotient	29.305	40	0.894
Number of valid cases	63		
a. 50 cells (92.6%) have an expected frequency of 5 or less. The smallest expected frequency is 0.03.			

106

Supp. Table 1

tumor score * EGFR staining intensity – contingency table							
number							
		EGFR staining intensity					total
		0	weak	intermediate	strong	missing	
valid	1	10	5	5	6	1	27
	2	5	3	6	2	1	17
	2a	0	0	1	1	0	2
	2b	1	0	1	0	0	2
	3	0	0	0	0	1	1
	3a	1	3	0	2	0	6
	3b	1	0	0	0	0	1
	4	0	0	0	1	0	1
	X	2	2	0	2	0	6
	total	20	13	13	14	3	63

chi-squared test			
	value	df	Asymptotic significance (two-sided)
Chi-squared (Pearson)	40.619[a]	32	0.141
Likelihood-Quotient	29.875	32	0.575
Number of valid cases	63		
a. 40 cells (88.9%) have an expected frequency of 5 or less. The smallest expected frequency is 0.05.			

tumor score * EGFR IRS – contingency table											
number											
		EGFR – IRS									total
		0	1	2	4	5	6	8	9	12	
valid	1	10	1	1	3	1	0	5	2	4	27
	2	5	0	3	2	1	1	3	0	2	17
	2a	0	0	0	0	0	1	0	0	1	2
	2b	1	0	0	0	0	1	0	0	0	2
	3	0	0	0	0	1	0	0	0	0	1
	3a	1	0	0	3	0	0	1	0	1	6
	3b	1	0	0	0	0	0	0	0	0	1
	4	0	0	0	0	0	0	0	0	1	1
	X	2	0	1	1	0	0	0	0	2	6
	total	20	1	5	9	3	3	9	2	11	63

chi-squared test			
	value	df	Asymptotic significance (two-sided)
Chi-squared (Pearson)	67.241[a]	64	0.367
Likelihood-Quotient	45.01	64	0.966
Number of valid cases	63		
a. 79 cells (97.5 %) have an expected frequency of 5 or less. The smallest expected frequency is 0.02.			

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E

Supp. Table 1

tumor grading					
		frequency	percentage	valid percentage	cumulated percentage
valid	no grading or missing	6	9.5	9.5	9.5
	Well-differentiated	11	17.5	17.5	27.0
	moderatly-differentiated	29	46.0	46.0	73.0
	poorly-differentiated	17	27.0	27.0	100.0
	total	63	100	100	

tumor grading * EGFR percentage of positive cells - contingency table								
number								
		EGFR percentage of positive cells						total
		negative	<10%	11-50%	51-80%	> 80%	missing	
valid	no grading or missing	1	1	1	0	3	0	6
	Well-differentiated	2	0	1	2	6	0	11
	moderatly-differentiated	10	1	3	2	12	1	29
	poorly-differentiated	7	0	0	1	7	2	17
	total	20	2	5	5	28	3	63

chi-squared test			
	value	df	Asymptotic significance (two-sided)
Chi-squared (Pearson)	13.227[a]	15	0.585
Likelihood-Quotient	14.112	15	0.517
Number of valid cases	63		
a. 20 cells (83.3%) have an expected frequency of 5 or less. The smallest expected frequency is 0.19.			

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Supp. Table 1

tumor grading * EGFR staining intensity – contingency table							
number							
		EGFR staining intensity					total
		0	weak	intermediate	strong	missing	
valid	no grading or missing	1	3	0	2	0	6
	Well-differentiated	2	2	6	1	0	11
	moderatly-differentiated	10	6	4	8	1	29
	poorly-differentiated	7	2	3	3	2	17
	total	20	13	13	14	3	63

chi-squared test			
	value	df	Asymptotic significance (two-sided)
Chi-squared (Pearson)	17.287[a]	12	0.139
Likelihood-Quotient	16.752	12	0.159
Number of valid cases	63		
a. 15 cells (75.0%) have an expected frequency of 5 or less. The smallest expected frequency is 0.29.			

tumor grading * EGFR IRS – contingency table											
number											
		EGFR – IRS									total
		0	1	2	4	5	6	8	9	12	
valid	no grading or missing	1	1	1	1	0	0	0	0	2	6
	Well-differentiated	2	0	1	1	0	2	4	0	1	11
	moderatly-differentiated	10	0	3	5	1	0	2	2	6	29
	poorly-differentiated	7	0	0	2	2	1	3	0	2	17
	total	20	1	5	9	3	3	9	2	11	63

chi-squared test			
	value	df	Asymptotic significance (two-sided)
Chi-squared (Pearson)	32.283[a]	24	0.12
Likelihood-Quotient	29.759	24	0.193
Number of valid cases	63		
a. 33 cells (91.7%) have an expected frequency of 5 or less. The smallest expected frequency is 0.10.			

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