**Generation of Stably Transduced Cell Lines**

Lentiviral vectors (~3 μg) were co-transfected with 2.25 μg psPAX2.0 plasmid (Addgene, catalog number: 12260) and 0.75 μg CMV VSV-G (Addgene, catalog number: 98286) packaging plasmid into 5 × 106 HEK293FT cells cultured on 10 cm plates in complete DMEM lacking antibiotic using the lipofectamine 2000 transfection reagent (Invitrogen). Plasmids were combined in 1 mL DMEM and mixed with 36 μL of lipofectamine reagent diluted in 1 mL DMEM and incubated for 30 minutes. DNA-Lipofectamine 2000 complexes were then added dropwise to 10 cm plates. ~16 hours post transfection, medium was exchanged with complete DMEM lacking antibiotics. Virus-containing supernatants were collected an additional 24 hours after medium exchange, filtered and aliquots were stored at -80 °C until use. The virus was used to infect target cells in 6-well plates in the presence of 6 μg/ml polybrene (Santa Cruz). 24 hours post-infection, fresh media was added to the target cells, which were allowed to recover for an additional 24 hours. Medium containing a predetermined concentration of the appropriate antibiotic for each cell line was then added, and transduced cells were selected for 3-7 days depending on the antibiotic used. Prior to viral transduction, virus was titered in each cell line to be transduced by crystal violet staining 5-7 days post antibiotic selection, using a predetermined concentration of the appropriate antibiotic puromycin, blasticidin, or geneticin. For generation of pLenti3.3TR expressing H460, H1792 and PC3 cells (referred to as H460‐3.3, H1792‐3.3, PC3‐3.3), virus was added at a MOI of 10. Clonal pLenti3.3TR expressing cells were selected by cloning cylinder and TetR expression was validated by western blotting with anti-Tetracyline Repressor antibody (MoBiTec, Göttingen, Germany, Cat. no. TET01). Tetracycline repressor expressing clones were then transduced with pLenti6.3 virus at an MOI of 1. Tetracycline inducible expression of genes of interest were assayed by western blotting after 24-48-hour exposure to 1 μg/mL tetracycline using anti-V5 antibody.

**Generation of Knockout Cell Lines and CRISPR Competition Assays**

HSV-control, PCNA, CPS1, and OXA1L gRNAs were cloned into U6-gRNA:ef1a-tGFP plasmid (Sigma-Aldrich, LV03) or U6-gRNA:ef1a-puro-2A-Cas9-2A-tGFP (Sigma-Aldrich, LV01) or purchased directly. For clonal knockout cell lines, virus produced from LV01 containing HSControl or target gRNA was added to H460 cells at an MOI of five and transduced cells were selected using puromycin containing medium. Individual clones were selected by cloning cylinder and knockout was validated by antibody and sanger sequencing using TIDE analysis1. The targeted genetic modifications in cell lines used in this study are summarized in Supplementary Table 7. For functional assays, at least three individual clones were evaluated from each gRNA. For competition assays, CAS9 expressing parental cell lines were generated for each cell line in the 10-cell line panel as described above using the CAS9BST plasmid (Sigma-Aldrich, CAS9BST) and blasticidin selection with a cell-line dependent concentration of blasticidin. CAS9 expression was validated by western blotting using anti-V5 tag antibody and CAS9 expressing cells were maintained in blasticidin supplemented medium. CAS9 expressing cells were plated on clear bottomed CellCarrier 96-well plates at 5000 cells per well in complete medium lacking phenol red/antibiotic and allowed to adhere overnight. The following day, 100 μL of titered gRNA virus was added to each well in the presence of 6 μg/ml polybrene to achieve ~50% transduction rate, which was validated by relative count of GFP expressing cells. 24 hours post transduction, plates were imaged on a CX5 high content imager using Thermo Scientific HCS Studio 4.0 Cell Analysis Software at 2x magnification using the 485/521 LED and brightfield light source.  GFP signal was used for autofocus and images were collected daily for five days post-transfection. Total GFP cell count was followed over time for each gRNA in triplicate and used to get a relative growth rate after normalization to HS-Control gRNAs. The essential gene PCNA was used as a positive control, which inhibited the growth of all cell lines tested.

HS-Control gRNA1: ACTGTTGACGGCGGCGATGT

HS-Control gRNA2: GCTGATACCGTCGGCGTTGG

PCNA gRNA1: CTACCGCTGCGACCGCAACC

PCNA gRNA2: GAGTATAAAATTGCGGATAT

PCNA gRNA3: AGCTGCACCAAAGAGACGT

PCNA gRNA4: CTCTGCAGGTTTACACCGC

CPS1 gRNA1: TTTTCATAGAGCTACCAATT

CPS1 gRNA2: CGTCTCAACATCTGAAACTC

CPS1 gRNA3: CCCTACCGTCTCCAGATCGA

OXA1L gRNA1: AATTGTAGCAGTGACTGCCAG

OXA1L gRNA2: ATGGCGATGGGACTAATGTG

OXA1L gRNA3: GTTTTCCAGTCGAATCAGAG

**Generation of Barcode Libraries and Barcoded Cell Lines**

Double stranded DNAs harboring random 20-mers were generated by hybridization and primer extension reaction on ice by mixing 6 μL of 10 μM oligo1, 6 μL of 10 μM oligo2, 10 μL of 10× PCR buffer without Mg2+, 3 μL of 50 mM MgCl2, 2 μL of 10 mM dNTP mix, 0.5 μL of Taq DNA polymerase, and 72.5 μL of H2O. Reactions were incubated at 94 °C for 5 min, cooled to 55 °C over the course of 10 minutes, and then heated at 72 °C for 30 minutes. Duplexes were precipitated by addition of 12.5 μL of 3 M sodium acetate and 250 μL of 100% ethanol followed by incubation overnight at -80 °C. Precipitated DNA was collected by centrifugation for 25 min at 20,000 × g, washed twice with cold 75% ethanol, and resuspended in 40 μL of H2O.  PLKO.1 vector (2 μg) was digested in 10 μL of CutSmart buffer, with 3 μL of Age-HF and 3 μL of EcoRI-HF in a 100 μL total reaction. Inserts were digested by mixing 40 μL of DNA duplexes, 20 μL of CutSmart buffer, 3 μL of Age-HF, 3 μL of EcoRI-HF, and 134 μL of H2O. Both reactions were incubated at 37 °C for 4 h. PLK0.1 digests were than purified by gel and DNA duplexes were precipitated with 200 μL of H2O and 400 μL of fresh Tris buffered phenol/chloroform/isoamyl alcohol (pH 8.0) for 20 minutes, and spun for 20 min at 20,000 × g. Liquid phase was transferred into a new tube, and 40 μL of 3 M sodium acetate and  1 mL of 100% ethanol was added and duplexes were precipitated overnight at -80 °C. DNA was collected by centrifugation for 25 min at 20,000 × g, washed twice with cold 75% ethanol, and resuspended in 10 μL of H2O. Ligation reactions were conducted by mixing 1 uL of precipitated duplex with 1 uL of a 25 ng/uL PLK0.1 digest, 1 μL of 10× T4 DNA ligase buffer, and 1 μL of T4 DNA ligase in a total volume of 10 μL and incubated for 4 hours at RT, followed by heat inactivation for 10 min at 65°C. Ligations were than transformed into STBL3, and plated onto ampicillin agar plates 10x10 cm and incubated overnight. Library preparation was validated by by PCR amplification and sanger sequencing of ten individual bacterial colonies by mixing 200 ng of DNA extracted from 5 mL bacterial culture with 2.5 μL of 10 μM oligo3, 2.5 μL of 10 μM oligo4, 10 μL of 5× Standard Phusion buffer, 1 μL of 10 mM dNTP mix, 0.5 uL Phusion polymerase and 31.5 μL of H2O and amplified using 35 cycles of the following sequence: 98°C 0:15, 98°C 0:10, 59°C 0:15, 72°C 0:30. PCR reactions were cleaned using a DNA clean and concentrator kit and submitted for sanger sequencing using oligo 5. After library validation, at least 106 colonies from specific duplex reactions were scraped into 1 mL LB and purified using a plasmid mini prep kit according to manufacturer’s instructions. Barcode libraries were packaged by transfecting low passage 293FT using PsPax2 and VSV-G plasmids using lipofectamine 2000. Virus was tittered by transducing H460 cells followed by selection with 2 μg/mL puromycin for two weeks and stained with crystal violet.  For final generation of barcoded H460 cells, 25% confluent H460 cells were transduced at an MOI of 0.05 in the presence of polybrene (6 μg/ml) in 6-well plates, followed by selection with 2 μg/mL puromycin for two weeks. Barcoded H460 cells were maintained in puromycin containing medium and passaged no more than ten times.

Oligonucleotide sequences

oligo1: 5′-ggaaaggacgaaacACCGGTT-3′

oliog2: 5′-cgagaattcNNNNNNNNNNNNNNNNNNNNAAAAAACCGGTgtttcgtcctttcc-3′

oligo3: 5′-ggaggcttggtaggtttaagaa-3′

oligo4: 5′-ggatctctgctgtccctgtaat-3′

oligo5: 5′-ggaggcttggtaggtttaagaa-3′.

pLKO.1 (Addgene 10878)

psPAX2 (Addgene 12260)

pMD2.G (Addgene 12259).