Supplemental Experimental Methods:

Data processing

Data were processed in a reproducible GenePattern pipeline and are provided on the Project Achilles portal: http://www.broadinstitute.org/achilles. Briefly, raw read counts per million were normalized to the total read depth for each replicate, then $\log_2$ transformed before removing failed replicates and sgRNAs that were low in the initial pDNA pool. Except when specifically utilized in a described analysis, shRNAs that had > 1 perfect match with a PAM site in the reference genome were also removed. A fold change was calculated per sgRNA and the median of non-targeting controls (n=1000) in the GeCKOv2 library were subtracted from each sgRNA to generate a CRISPR guide score. Given the gene-independent effect of CRISPR-Cas9 described in this manuscript, we chose to use the second-best CRISPR-Cas9 guide score for the purpose of ranking gene-level dependencies in individual cell lines. This approach allowed demonstration of gene dependency while still displaying the results of individual sgRNA activity. For analyses comparing multiple genes in multiple cell lines, we accounted for differences in Cas9 activity/efficacy across cell lines using a cell-line specific Z-score, and then calculated composite CRISPR gene scores using the ATARiS method with a p-value threshold of 0.05, a previously described method to calculate gene-level dependency scores in shRNA data (1). RNAi data analyses performed in Fig. 2 and S1 were performed with pooled shRNA data and calculated using DEMETER (data not shown).

Analysis of published CRISPR-Cas9 screening data

Bayes Factor (BF) values were derived from Hart et al. (2), and fitness genes were determined per cell line according to the thresholds described therein. Gene level copy number data for HCT116, A375 and DLD1 were downloaded from the Cancer Cell Line Encyclopedia (http://www.broadinstitute.org/ccle/downloadFile/DefaultSystemRoot/exp_10/ds_20/CCLE_copynumber_byGene_2013-12-03.txt?downloadff=true&fileId=17598). Gene level copy number data for HeLa were downloaded from the Gene Expression Omnibus

(GEO) database, accession number GSE8605 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE8605). Threshold values of 0.3 and -0.3 were set for gains and losses, respectively. For the patient-derived glioblastoma cell line GBM514, copy number data were not available; however, GBM cell lines commonly exhibit a trisomy of chromosome 7 and a monosomy of chromosome 10 (3). An e-karyotyping analysis (4) revealed that these predicted copy number changes were indeed reflected by the gene expression profile of this cell line (2). Therefore, in this cell line the genes on chromosome 7 were determined as gained genes, and the genes on chromosome 10 were determined as non-gained genes. For each cell line, the statistical significance of the difference between average BF values of gained vs. non-gained genes was determined by a two-tailed Student's t-test. For each cell line, the statistical significance of the hit rate differences between gained, neutral and lost genes was determined by a Chi-squared test of independence with Yates correction. Similarly, for each cell line, the statistical significance of the hit rate difference between chromosome 7 and chromosome 10 was determined by a Chi-squared test of independence with Yates correction.

High-content imaging assay and analysis for cell cycle and DNA damage

PANC1 and CAL120 cells constitutively expressing Cas9 protein were plated at a density of 4000 cells per well in 96-well, black-wall, clear-bottom plates (Corning) and allowed to adhere for 24 hours. Replicate cell plates were infected with 10uL of BRD003 sgRNA-expressing lentivirus in 4ug/mL polybrene and centrifuged for 30 minutes at 2250 RPM.

48 hours post-infection a media change was conducted on all plates and replicate plates were labelled with 5uM Click-iT EdU (C10356, Thermo Fisher Scientific) for 30 minutes at 37C prior to fixation with 4% paraformaldehyde (PFA) for 20 minutes at RT. Two additional replicate plates, one administered 2ug/mL puromycin and the other plain media, were used to calculate infection efficiency based on a Cell-Titer-Glo readout.

To fluorescently label cell cycle components, fixed cells were first permeabilized with 0.5% Triton X100 in PBS for 20 minutes. Next, cells were washed twice with PBS and EdU incorporation was detected with AF647 using a Click-iT EdU labeling kit (C10356, Thermo Fisher Scientific). After washing twice with PBS, cells were then incubated for 30 minutes at RT in blocking solution (X090930-2, DAKO) and then incubated for 16 hours at 4C with a anti-pHH3 (S10) primary antibody (Rabbit: #9701, Cell Signaling, 1:800) and a anti-phospho-histone H2A.X (Ser139) primary antibody (Mouse: 05-636, END Millipore, 1:1250) diluted in antibody dilution buffer (K800621-2, DAKO). Post primary antibody incubation, cells were washed twice with 0.05% Tween20 in PBS before incubation for 2 hours at RT with a cocktail consisting of anti-mouse AF568 secondary antibody (Goat: A-11004, Thermo Fisher Scientific, 1:500), anti-rabbit AF488 antibody (Goat: A-11008, Thermo Fisher Scientific, 1:500) and Hoechst 33342 (H3570, Thermo Fisher Scientific, 1ug/mL) diluted in antibody dilution buffer. Cells were subsequently washed twice with 0.05% Tween20 in PBS. After two additional washes with PBS the cell plates were imaged with the OperaPhenix imaging system on 20x magnification.

Images were analyzed using the PerkinElmer Harmony software. The non-border nuclei used for cell cycle analysis were gated based on their roundness. Cell cycle phase for each cell was identified using a proper threshold for Hoechst 33342 integral staining (DNA content); Hoechst 33342 Maximal staining (DNA condensation); EdU mean staining (S-phase); and pHH3 (S10) mean staining (M-phase). The values for the thresholds were identified using scatter plots created in Spotfire. DNA damage was analysed using gH2AX staining as an average number of foci per area of non-border nucleus.

Analysis Sequence

Images were analyzed using the PerkinElmer Harmony software. The non-border nuclei used for cell cycle analysis were gated based on their roundness. Cell cycle phase for each cell was identified using a proper threshold for Hoechst 33342 integral

staining (DNA content); Hoechst 33342 Maximal staining (DNA condensation); EdU mean staining (S-phase); and pHH3 (S10) mean staining (M-phase). The values for the thresholds were identified using scatter plots created in Spotfire. DNA damage was analysed using γ-H2AX staining as an average number of foci per area of non-border nucleus.

Building blocks for the analysis pipeline are defined as follows:

1. [Input Image]– Individual Planes, FFC Basic
2. [Find Nuclei]– Channel: HOECHST 33342; Method: M; Output Population: Nuclei
3. [Select Population]– Population: Nuclei; Method: Common Filters (Remove Border Objects); Output Population: Nuclei Selected
4. [Calculate Morphology Properties]– Population: Nuclei Selected; Region: Nucleus; Method: Standard (Roundness)
5. [Calculate Intensity Properties (1)]– Channel: HOECHST 33342; Population: Nuclei Selected; Region: Nucleus; Method: Standard (Sum; Maximum); Output Properties: DNA
6. [Calculate Intensity Properties (2)]– Channel: AF647; Population: Nuclei Selected; Region: Nucleus; Method: Standard (Mean); Output Properties: EdU
7. [Calculate Intensity Properties (3)]– Channel: AF488; Population: Nuclei Selected; Region: Nucleus; Method: Standard (Mean); Output Properties: pHH3 (S10)
8. [Find Spots]- Channel: AF568, Population: Nuclei Selected; Region: Nucleus; Method: A; Output Properties: gH2AX Spots
9. [Select Population (1)]- Population: Nuclei Selected; Method: Filter by Property; Properties: Intensity Nucleus Hoechst 3342 Sum, Nucleus Roundness; Output Population: Nuclei for Cell Cycle
10. [Select Population (2)]- Population: Nuclei for Cell Cycle; Method: Filter by Property; Properties: Intensity Nucleus AF647 Mean, Intensity Nucleus Hoechst Sum, Intensity Nucleus Hoechst Maximum, Intensity Nucleus AF488 Mean: Output Population: G1, G2, EdU Positive, M

11. [Define Results (1)]- Population: Nuclei selected: Number of Spots per Area of Nucleus Mean
12. [Define Results (2)]- Population: Nuclei for cell cycle: Number of Spots per Area of Nucleus EdU Positive Mean, G1 Mean, G2 Mean, M Mean

Computational Analyses

Computational analyses were performed using R/Bioconductor (https://www.R-project.org, https://www.bioconductor.org). *TP53* mutation status was curated from the Cancer Cell Line Encyclopedia and COSMIC and is indicated in Table S1.

References

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4. Ben-David U, Mayshar Y, Benvenisty N. Virtual karyotyping of pluripotent stem cells on the basis of their global gene expression profiles. Nature protocols. 2013;8:989-97.