Supplemental Figure Legends

Figure S1. Genome-scale CRISPR-Cas9 screening identifies a strong correlation between copy number and sensitivity to CRISPR-Cas9 genome editing. Data are shown for three additional amplified loci: Chromosome 19q in PANC-1 (A, D), Chromosome 12 multi-focal in CAL120 (B, E), and Chromosome 22q in K562 (C-F). (A-C) Two tracks are plotted along genomic coordinates within the region defined the red box on the chromosome schematic. Top: ABSOLUTE genomic copy number from Cancer Cell Line Encyclopedia (CCLE) SNP arrays with red indicating copy number gain above average ploidy and blue indicating copy number loss below average ploidy; Bottom: CRISPR-Cas9 guide scores plotted according to the 2^{nd} most dependent sgRNA for each gene with purple trend line indicating the mean CRISPR-Cas9 sensitivity for each copy number segment defined from the above track. The driver oncogenes *AKT2* (A), *CDK4* (B) and BCR (C) are highlighted in orange. (D-F) Boxplots of CRISPR-Cas9 guide scores for both un-expressed and expressed genes located on (red) or off (black) of each represented amplicon. For CAL120, the amplicon represented in panel E red box plots ranges from 57.9-71.2 Mb on the corresponding plot in panel B. The number of represented genes is noted above each box plot.

Figure S2. Global summary of the relationship of CRISPR-Cas9 guide scores to genomic copy number for all 33 cell lines screened. For each copy-number-defined genomic segment, median CRISPR-Cas9 guide score is plotted against copy number. Each circle represents a single genomic segment of defined copy number for the indicated cell line. The size of the circle corresponds to the number of sgRNAs targeting that segment. Non-targeting negative control sgRNAs are shown with a blue boxplot and known cell essential genes (defined as positive controls) are shown as a red boxplot, both embedded within the plot.

Figure S3. Amplified genes represent the strongest perceived dependencies in pooled CRISPR-Cas9 screening data. Waterfall plots showing CRISPR-Cas9 guide scores for all genes in the pooled screens performed on the indicated cell lines for the amplicons shown in Figure S1: PANC-1 Chromosome 19q, CAL120 Chromosome 12p and K562

Chromosome 22. Rank ordered plots showing the second-best CRISPR-Cas9 guide score for each gene in the indicated cell lines. sgRNAs targeting genes within the amplicons represented in Figure S1 are highlighted in red for PANC-1 19q amplicon (A), CAL120 12p amplicon (B) and K562 22q amplicon (C). These amplicon-targeting sgRNAs are significantly enriched as apparent dependencies relative to the other sgRNAs targeting genes outside these amplicons (one-sided Kolmogorov–Smirnov test: p = 2.08E-33, A; p = 3.68E-23, B; p = 8.62E-34, C)

Figure S4. Evaluation of the influence of copy number on CRISPR-Cas9 dependency scores in published data from Hart et al. (25) (A, B) Plot of median CRISPR-Cas9 guide score against copy number for two cell lines screened by Hart et al, HeLa (A) and HCT116 (B). Each circle represents a single genomic segment of defined copy number for the indicated cell line. The size of the circle corresponds to the number of sgRNAs targeting that segment. Non-targeting negative control sgRNAs are shown with a blue boxplot and known cell essential genes (defined as positive controls) are shown as a red boxplot embedded within the plot. (C) Gained genes tend to have higher Bayes Factor (BF) values compared to non-gained genes, across all CRISPR screens. This trend is not observed in the shRNA screen of HCT116. Significance was determined by two-tailed Student's t-test. *, $p<1e10^{-3}$; n.s., $p>5e10^{-2}$. (D) In the CRISPR-Cas9 screen (but not in the shRNA screen) performed with the HCT116 cell line, the hit rate of gained genes is significantly higher than that of neutral genes, which is in turn significantly higher than that of lost genes. Significance was determined by a Chi-squared test of independence. *, $p=1e10^{-4}$, n.s., $p>5e10^{-2}$. (E) The relative number of hits on chromosome 7 compared to chromosome 10 is skewed in the GBM514 cell line, which is predicted to harbor a trisomy of chromosome 7 and a monosomy of chromosome 10. Significance was determined by a Chi-squared test of independence. *, $p=1.8e10^{-3}$, n.s., $p>5e10^{-2}$.

Figure S5. Representative examples of structural variations leading to copy number amplification underlying the gene-independent anti-proliferative response to CRISPR-Cas9 targeting of loci within these regions. For each example, data shown include copy

number and rearrangement data from whole genome sequencing (top) and CRISPR-Cas9 screening data (bottom). CRISPR-Cas9 guide scores plotted according to the 2nd most dependent sgRNA for each gene with purple trend line indicating the mean CRISPR guide score for each copy number defined segment. A) Tandem duplication. A copy-number plot of chromosome 8 in HT29 large intestine cancer cell line. The read depth data are normalized by the median coverage in the 8p arm. The rearrangement generating the amplicon in 8q is a tandem duplication. The large copy-number difference between the amplicon (~9) and the flanking regions (~3) indicates that the sequence is duplicated twice, generating three extra copies. B) Inter-chromosomal translocation. A copy-number plot of chromosome 17 in the CAL120 breast cancer cell line. The read depth data are normalized by the median coverage in the 17p arm. The 17q-ter amplification is translocated to chromosome 5. The CRISPR-Cas9 screening data show a small but appreciable reduction in mean CRISPR-Cas9 guide score for the region of copy number gain defined by WGS, consistent with the CRISPR-CN effect occurring even at low level copy number gains.

Figure S6: Representative example of complex amplified locus showing a CRISPR-CN correlation. Data shown include copy number and rearrangement data from whole genome sequencing (top) and CRISPR-Cas9 screening data (bottom). CRISPR-Cas9 guide scores plotted according to the 2nd most dependent sgRNA for each gene with purple trend line indicating the mean CRISPR-Cas9 guide score for each copy number defined segment. Copy-number plot of a focal amplification at 19q in PANC-1 pancreatic cancer cell line showing a complex locus with breakage-fusion-bridge (BFB) cycles and chromothripsis. The read depth data are normalized by the flanking regions. The rearrangement pattern indicates that this region first underwent multiple breakage-fusion-bridge cycles, generating a high-level focal amplification that encompasses AKT2 (40.74-40.79 Kb). The BFB amplification subsequently underwent a chromothripsis event that generated interspersed deletions in the amplified region.

Figure S7. Comparative analysis of the relationship of CRISPR-Cas9 guide scores to the predicted number of CRISPR-Cas9-induced DNA cuts based on either copy number

or total predicted perfect-match on- and off-target alignments. Data is shown for all 33 cell lines screened. CRISPR-Cas9 guide scores are shown on the Y-axis and the predicted number of DNA cuts is shown on the X-axis. sgRNAs are divided into three groups. In red are sgRNAs that target a single locus, and therefore total number of predicted cuts is based on copy number. In yellow are sgRNAs that target multiple loci within a single chromosome (intra-chromosomal). In blue are sgRNAs that target multiple loci across multiple chromosomes (inter-chromosomal). A more potent detrimental influence on cell viability is observed for multiple CRISPR-Cas9-induced DNA cuts across multiple chromosomes (inter-chromosomal) as compared to those restricted to a single chromosome (intra-chromosomal).

Figure S8. Comprehensive analysis of CRISPR-Cas9 sensitivity correlated to the total predicted number of DNA cuts conferred by each sgRNA. (A, B) Representative plots for PA-TU-8902 (A) and Panc 08.13 (B) cell lines showing estimated number of DNA cuts (X-axis) conferred by each sgRNA in the screen and the corresponding impact on CRISPR guide score. Each point represents a single sgRNA in the screen. Distributions of the negative non-targeting controls (blue) and the cell essential ("positive control") genes (red) are shown at right. For each bin of data points with a defined estimated number of cuts, the density of data points is depicted using color ranging from blue (least dense) to red (most dense), and the median sensitivity value is shown as a black hash mark. A segmented, least-squares model is fit to this data with an inflection point to model the observed plateau, or "floor," in CRISPR-Cas9 guide scores with increasing estimated number of DNA cuts, thus highlighting the limited resolution of CRISPR-Cas9 screening for differentiating sgRNAs conferring multiple DNA cuts past certain threshold. The slope of the first segment ("CRISPR-Cut Index", CCI-Total) models the cell line-specific impact of increasing number of DNA cuts on sensitivity to CRISPR-Cas9 targeting. (C) Cross cell line correlation of the CCI-Total with the median CRISPR guide score of positive controls, which models the per cell line efficacy of CRISPR-Cas9 genome editing. R2 = 0.4723 for linear regression. (D) Box plots or CCI-Total binned by TP53 mutation status (WT = wild-type) and (Mut = mutant), as curated per Table S1. Two-tailed Student's T-test: t = -5.9615, df = 31, p-value = 1.369e-06.

Figure S9. (A) PANC-1 infection efficiency corresponding to Figure 6 *in vitro* validation experiment measuring short-term proliferation and viability response of PANC-1 cells transduced at high multiplicity of infection. sgRNA order and color scheme are as indicated in Fig. 6. Cell-Titer-Glo was performed at 6 days post-infection in wells with and without puromycin selection to obtain infection efficiency. (B) Immunoblot of protein from PANC-1 cells harvested 48 hours after infection at high MOI with the indicated sgRNAs targeting inside (19q8) or outside (19q4) the PANC-1 19q13 amplicon shown in Fig. 6A. Multi-targeting sgRNAs (MT-1 and MT-2), as well as negative control sgRNA LUC-1 are also shown. 5-fluorouracil (5-FU, 500 nM x 16 hr) treated and uninfected controls are indicated.

Figure S10. CRISPR-Cas9 targeting of amplified regions or multiple genomic loci induces DNA damage and a G2 cell cycle arrest in CAL120 cells. (A) Schematic of the CAL120 12q amplicon demonstrating ABSOLUTE DNA copy number (top panel) and CRISPR guide scores (middle panel) mapped by genomic position. Schematic and color scheme are similar to that detailed in Fig. 2. (B) *In vitro* validation experiment measuring arrayed proliferation and viability response of CAL120 cells at 6 days post-infection with sgRNAs targeting regions inside (red) and outside (blue) of the demonstrated amplicon. sgRNAs targeting intergenic regions are labeled by chromosomal locus and columns are given a checkered pattern. Multi-targeted sgRNAs (MT-1 and MT-2) are indicated by black bars. sgRNAs targeting an alternative unamplified locus (19q, orange) and known essential genes (green) are also shown. Non-targeting negative control sgRNAs are shown in yellow. Dots placed below the copy number panel correspond to the validation sgRNAs targeting the indicated genes or intergenic regions on the locus, and are matched by color and left-to-right genomic position. Cell-Titer-Glo was performed at 6-days post-infection. Error bars indicate SD of biologic replicates (n=3). $p < 0.0001$ for two-tailed T-test comparing sgRNAs inside (red) vs outside (blue and orange) the amplicon. (C) Infection efficiency corresponding to each sgRNA used in panel (B). (D) Plot of the percentage of CAL120 cells in each phase of the cell cycle at 48 hours post-infection with the indicated sgRNAs targeting inside (red) or outside (blue) the amplicon.

Data for multi-targeted sgRNAs (MT-2) or control sgRNA's targeting an alternative locus (22q-1) or control genes are also shown. Percentage of cells in each phase of the cell cycle is indicated by a unique pattern within the column corresponding to each cell cycle phase. Colors scheme is as indicated above, with coloration of the G2 and S phases for emphasis. Error bars represent the standard deviation for the mean of three replicates. (E) Plot of the number of γ-H2AX foci present in CAL120 cells at 48 hours post-infection with the indicated sgRNAs. Color scheme as indicated above, with checkered pattern corresponding to sgRNAs targeting intergenic regions.

Table S1: Cell line sample information

Table S2: Positive control KEGG gene sets

Table S3: sgRNA's utilized in validation experiments