Agreement between two large pan-cancer CRISPR-Cas9 gene dependency data sets

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Genome-scale CRISPR-Cas9 viability screens performed in cancer cell lines provide a systematic approach to identify cancer dependencies and new therapeutic targets. As multiple large-scale screens become available, a formal assessment of the reproducibility of these experiments becomes necessary. We analyze data from recently published pan-cancer CRISPR-Cas9 screens performed at the Broad and Sanger Institutes. Despite significant differences in experimental protocols and reagents, we find that the screen results are highly concordant across multiple metrics with both common and specific dependencies jointly identified across the two studies. Furthermore, robust biomarkers of gene dependency found in one data set are recovered in the other. Through further analysis and replication experiments at each institute, we show that batch effects are driven principally by two key experimental parameters: the reagent library and the assay length. These results indicate that the Broad and Sanger CRISPR-Cas9 viability screens yield robust and reproducible findings.
Results
Overview of data sets and comparison strategy. We compared two sets of pooled genome-scale CRISPR-Cas9 drop out screens in cancer cell lines, generated at the Broad Institute and the Sanger Institute through independently designed experimental pipelines (detailed in Fig. 1a, Supplementary Data 1 and Supplementary Methods), considering 147 cell lines and 16,733 genes screened independently by both institutes (Supplementary Data 2). We performed comparisons of individual gene scores, quantifying the reduction of cell viability upon gene inactivation via CRISPR-Cas9 targeting; of profiles of such scores across cell lines (gene dependency profiles); of profiles of such scores across genes in individual cell lines (cell line dependency profiles).

We calculated gene scores using three different strategies. First, we considered fully processed gene scores, available for download from the Broad Institute and Sanger Institute through independently designed experimental pipelines of single-guide RNAs (sgRNAs) being performed on growing numbers of cancer in vitro models14-16. The output of these screens can be used to identify and prioritize new cancer therapeutically targets13. However, fully characterizing genetic vulnerabilities in cancers is estimated to require thousands of genome-scale screens14,15.

We present a comparative analysis of data sets derived from the two largest independent CRISPR-Cas9 based gene-dependency screening studies in cancer cell lines published to date13,15,16, part of the Cancer Dependency Map effort17,18. The analysis aims to assess the concordance of these data sets and that of the analytical outcomes they yield when investigated individually. To this aim, our computational strategy includes comparisons at different levels of data-processing and abstraction: from gene-level dependencies to molecular markers of dependencies, and genome-scale cell line profiles of dependencies. Lastly, we shed light on the differences in the experimental settings that give rise to batch effects across independent studies of this kind, discerning between biological and technical confounding factors.

Agreement of selective gene score profiles across cell lines. In both studies, most genes show little variation in their scores across cell lines. Thus we expect low shared variance even if most scores are numerically similar between the data sets20. Accordingly, we focused on a group of genes for which the score variance across cell lines of potential biological interest. These are genes whose dependency profile suggests a strong biological selectivity in at least one of the two unprocessed data sets, identified using the Likelihood Ratio Test (NormLRT) test introduced in McDonald et al.21. We call these 49 genes Strongly Selective Dependencies (SSDs) (Supplementary Data 4). We evaluated the agreement between gene score patterns across cell lines using Pearson’s correlations to test the reproducibility of selective viability phenotypes. Figure 2a illustrates the score patterns for the example cancer genes MM4 (R = 0.820, beta test p = 6.91 × 10^{-37}), KRAS (R = 0.765, p = 1.66 × 10^{-29}), CTNB1 (R = 0.803, p = 1.92 × 10^{-34}), and SMARCA4 (R = 0.664, p = 4.61 × 10^{-20}) with unprocessed data (N = 147). For SSDs and unprocessed data, the median correlation was 0.633 and 84% of SSDs showed a correlation greater than 0.4. Five SSDs showed a correlation below 0.2 (ABHD2, CDC62, HIF1A, HSPA5, C17orf64), and are discussed further below. As expected, correlation across data sets for all genes was lower (median R = 0.187, 8.34% genes with R > 0.4).

One important use of these screens is to consistently classify cells as dependent or not dependent on selective dependencies. Therefore, we evaluated the agreement of the Broad and Sanger data sets on identifying cell lines that are dependent on each SSD gene. We classified cell lines as dependent on a given gene if its gene score represents a false discovery rate (FDR) less than 0.05 (see the Methods section). Genes scores with greater than 5% FDR are dominated by a large group of scores near zero (Fig. 2c).

The area under the receiver-operator characteristic (AUROC) for recovering binary Sanger dependency on SSDs using Broad gene scores was 0.940 in processed data, 0.963 in unprocessed data, and 0.971 in corrected data; to recover Broad binary dependency from Sanger scores, AUROC scores were 0.918, 0.870, and 0.968 respectively. The recall of Sanger-identified gene scores between the two data sets can be considered a function of two variables: the mean dependency across all cell lines for each gene (relevant to infer common dependencies), and the patterns of scores across cell lines for each gene (relevant to predict selective oncology therapeutic targets). Mean gene scores among all cell lines showed excellent agreement (Supplementary Fig. 1a), with Pearson correlation = 0.784 and 0.818, respectively for processed and unprocessed data (p below machine precision in both cases using SciPy’s beta distribution test; N = 16,773). The effect of ComBat correction on our data is to align gene means and variances (Supplementary Fig. 1b). As expected, after ComBat correction the Pearson correlation of gene means was = 0.9997, and the correlation of gene standard deviations (SDs) was = 0.957.

We further tested whether it was possible to recover consistent sets of common dependencies. To this end, we defined as common dependencies those genes that rank among the top dependencies when considering only their 90th percentile of least dependent cell lines, with the score threshold for top dependencies determined by the local minimum in the data (Fig. 1c). For the unprocessed data, the Broad and Sanger jointly identify 1,031 common dependency genes (Supplementary Data 3). 260 putative common dependencies were only identified by the Sanger and 397 were only identified by the Broad (Cohen’s kappa = 0.737, Fisher’s exact test p-value below machine precision, N = 16,773, Fig. 1d).
dependent cell lines in Broad data was 0.781 with precision equal
to 0.255 for processed data, 0.775 and 0.258 for unprocessed data,
and 0.754 and 0.587 for batch-corrected data (Supplementary
Fig. 1c). Agreement is higher than could be expected by chance
under all processing regimes (Fisher’s exact test $p = 8.99 \times 10^{-43}$
in processed, $9.65 \times 10^{-44}$ in unprocessed, and $5.29 \times 10^{-198}$
in batch-corrected data; $N = 7,203$). A large proportion of Broad-
exclusive dependent cell lines (53.4% in processed data and 47.7%
in unprocessed data) were due to the single gene HSPA5, which is
an SSD in Sanger data but a common dependency in Broad data.
Fig. 2 Reproducibility of gene and cell line dependency profiles. a Examples of gene score pattern comparisons for selected known cancer genes. b Distribution of correlations of scores for individual genes in unprocessed data. c Gene scores for strongly selective dependencies across all cell lines, with the threshold for calling a line dependent set at an FDR of 0.05. d tSNE visualization of cell lines in unprocessed data based on the correlation between cell line profiles of gene scores. Colors represent the cell line while shape denotes the study of origin. e The same as in (d) but for data batch-corrected using ComBat. f Recovery of a cell line’s counterpart in the other data set before (Uncorrected) and after correction (Corrected). Value on the y-axis shows percentages of cell lines whose matching counterpart in the other data set is within its k-nearest cell lines, i.e. the k-neighborhood on the x-axis, based on a Pearson correlation distance metric. nAUC values are shown in brackets. Three different gene sets were considered to calculate the correlation between cell lines. First, using all genes (uncorrected and corrected all), second, using genes that are dependencies for at least one cell line (corrected variable) and third, using strongly selective dependencies (corrected SSD) genes.
Examinaing SSDs individually, we found median Cohen’s kappa for sensitivity to individual SSDs of 0.461 in processed, 0.609 in unprocessed, and 0.758 in batch-corrected data. In unprocessed data, 59.2% of SSDs had Cohen’s kappa greater than 0.4, as opposed to 0.03% expected by chance (Supplementary Fig. 1c).

**Agreement of cell line dependency profiles.** Previous literature on reproducibility highlighted the importance of considering agreement along both the perturbation and cell line axes of the data. We assembled a combined data set of cell line dependency profiles from both studies and computed all possible pairwise correlation distances between them, using genes that were dependencies in at least one cell line (variable genes). A t-distributed stochastic neighbor embedding (tSNE) visualisation derived from these distance scores is shown in Fig. 2d. For the uncorrected data, we observed a perfect clustering of the dependency profiles by their study of origin, confirming a major batch effect. However, following batch correction, we observed integration between studies and increased proximity of cell lines from one study to their counterparts in the other study (Fig. 2e). To quantify agreement, for each cell line dependency profile in one data set, we ranked all the others (from both data sets) based on their correlation distance to the profile under consideration. For batch-corrected data, 175 of 294 (60%) cell line dependency profiles from one study have their counterpart in the other study as the closest (first) neighbor, and 209 of 294 (71%) of cell lines have it among the five closest neighbors (area under the normalized Recall curve — nAUC — averaged across all profiles = 0.91 for batch-corrected data, and 0.53 for uncorrected data, Fig. 2f). Similar results were obtained across dependency profiles restricted to different sets of genes, with the best performance obtained when considering SSD genes only (nAUC = 0.94) and worst performances when considering all genes (nAUC = 0.90).

The percentage of cell lines matching closest to their counterparts in the other study was 57% when considering all genes and 43% when considering SSD genes. Further, the tSNE plots for each tested gene set showed similar improvement after correction (Supplementary Fig. 2a–b).

The batch correction also aligned numbers of significant (at 5% FDR) dependencies across cell lines between the two data sets (median number of dependencies 2,109 and 1,717 before, and 2,053 and 1,950 after correction, for Broad and Sanger respectively, Supplementary Fig. 3a). The average proportion of dependencies detected in both studies over those detected in at least one study also increased across cell lines from 47.75% to 59.14%. Furthermore, the correlation between cell lines after correction rose above the correlation within each individual screen for each gene set considered (Supplementary Fig. 3b). We finally examined whether the residual disagreement in corrected data might be related to screen quality and if there are tissues for which corresponding cell lines showed a consistently higher/ lower agreement across the two studies. We assessed screen quality by computing true positive rates (TPRs) for recovering common essential genes in each cell line with a fixed 5% FDR, determined from the distribution of nonessential genes in the cell line. We found that mean screen quality is a strong predictor of screen agreement for both the uncorrected and batch-corrected data sets (t-test p-values 2.06 × 10^{-33}, 4.74 × 10^{-35}, N = 147 and adjusted R-squared 0.65, 0.64 for uncorrected and batch-corrected respectively; Supplementary Fig. 3c). In addition, we observed no differences in screen agreement when stratifying cell line based on their tissue of origin (Supplementary Fig. 3d), with screen quality being highly correlated with screen agreement invariantly across tissues (Supplementary Fig. 3e and Supplementary Data 5).

**Agreement of gene dependency biomarkers.** A selective dependency is of limited therapeutic value unless it can be reliably associated with an informative molecular feature of cancer (biomarker). Following a similar approach to that presented by the Cancer Cell Line Encyclopedia and Drug Sensitivity in Cancer consortia, we performed a systematic test for molecular-feature/dependency associations on the two data sets. To this aim, we considered a set of Cancer Functional Events consisting of 578 molecular features selected in Iorio et al. and based on their clinical relevance and encompassing mutations in high-confidence cancer driver genes, amplifications/deletions of chromosomal segments recurrently altered in cancer, hypermethylated gene promoters, microsatellite instability status, and the tissue of origin of the cell lines (Supplementary Data 5). We considered each of these features in turn and observed its status in the cell lines screened at both Sanger and Broad. Based on this, cell lines were split into two groups (respectively with negative/positive feature) and each of the SSDs was t-tested for significant differences in gene scores across the obtained two groups of cell lines.

These tests yielded 71 out of 29,350 possible significant associations (FDR < 5%, ΔFC < −1) between molecular features and gene dependency when using the Broad unprocessed data, and 90 when using the Sanger unprocessed data (Supplementary Data 6). Of these, 55 (77% of the Broad associations and 61% of the Sanger ones) were found in both data sets (FET p-value = 9.08 × 10^{-133}, Fig. 3a and Supplementary Data 6). The concordance between the associations identified by each study was proportional to the threshold used to define significance (Supplementary Data 7). This was assessed by first considering the associations found significant (FDR < 5%) in one study as positive controls and calculating precision, recall, and sensitivity using a rank predictor based on the p-values obtained in the other study for all associations. We then tested how performance changed when considering increasingly stringent subsets of significant associations as positive controls and found that the most significant associations in one study were the most likely to be recovered in the other (Fig. 3b). Further, the overall correlation between differences in gene depletion FCs between cell lines with and without a specified molecular feature was equal to 0.763, and 99.2% of associations had the same sign of differential dependency across the two studies (Fig. 3a). This indicates that the studies agree not only on the existence of specific biomarkers but also on their robustness.

Gene dependency associations identified with both data sets included expected as well as potentially novel hits. Examples of expected associations included increased dependency on **ERBB2** in **ERBB2**-amplified cell lines, increased dependency on beta-catenin in APC mutant cell lines and increased dependency on **MYCN** in peripheral nervous system cell lines. A potentially novel association between **FAM72B** promoter hypermethylation and beta-catenin was also consistently identified across data sets (Fig. 3c).

We also considered gene expression to mine for possible biomarkers of gene dependency using RNA-seq data sets maintained at Broad and Sanger institutes. To this aim, we considered as potential biomarkers 1,987 genes from intersecting the top 2,000 most variable gene expression levels measured by either institute. Clustering the RNA-seq profiles revealed that each cell line’ transcriptome matched closest to its counterpart from the other institute (Supplementary Fig. 4a).

We correlated the gene expression level for the most variably expressed genes to the gene dependency profiles of the SSD genes. Systematic tests of each correlation identified significant associations between gene expression and dependency. Further, as with the genomic biomarkers, we found significant overlap between gene expression biomarker associations identified in each data set.
with 4,459 (52% of Broad and 66% of Sanger gene expression biomarkers) found significant for both studies, out of 97,363 tested (Fisher’s exact test p-value below machine precision), and strong overall agreement of correlation scores between gene expression markers and SSD genes dependency across data sets (Pearson’s correlation 0.804, Fig. 3d). We observed both positive and negative correlations consistently across data sets; for example, ERBB2 gene score was positively correlated with its expression, while ATP6V0E1 showed significant dependency when its paralog ATP6V0E2 had a low expression (Fig. 3e).
Elucidating sources of disagreement between the two data sets.

Despite the concordance observed between the Broad and Sanger data sets, we found batch effects in the unprocessed data both in individual genes and across cell lines. Although the bulk of these effects are mitigated by applying an established correction procedure, their cause is an important experimental question. We conducted gene set enrichment analysis of genes sorted according to the loadings of the first two principal components of the combined unprocessed gene scores using a comprehensive collection of 186 KEGG pathway gene sets from Molecular Signature Database (MsigDB). We found significant enrichment for genes involved in spliceosome and ribosome in the first principal component, indicating that screen quality likely explains some variability in the data (Supplementary Fig. 5a, b). We then enumerated the experimental differences between data sets (Fig. 1a) to identify likely causes of batch effects. The choice of sgRNA can significantly influence the observed phenotype in CRISPR-Cas9 experiments, implicating the differing sgRNA libraries as a likely source of batch effect. Additionally, previous studies have shown that some gene inactivations result in cellular fitness reduction only in lengthy experiments. Accordingly, we selected the sgRNA library and the time point of viability readout for primary investigation as causes of major batch effects across the two compared studies.

To elucidate the role of the sgRNA library, we examined the data at the level of individual sgRNA scores. The correlation between fold change patterns of reagents targeting the same gene (co-targeting) across studies was related to the selectivity of that gene’s dependency (as quantified by the NormLRT score, Fig. 4a): a reminder that most co-targeting reagents show low correlation because they target genes exerting little phenotypic variation. However, even among SSDs there was a clear relationship between sgRNA correlations within and between data sets (beta test $p = 4.9 \times 10^{-10}$, N = 49; Fig. 4b). In particular, we note that the five SSDs (ABHD2, CDC62, HIF1A, HSPA5, C17orf64) identified earlier as having poor agreement between data sets have poor sgRNA correlation within data sets, thus indicating that this metric can be used to assess the reliability of a selective dependency.

One possible explanation of gene score disagreement is that sgRNAs in one of the two data sets had poor on-target efficacy. To identify such cases, we need an independent assessment of sgRNA efficacy. We estimated the efficacy of each sgRNA in both libraries using Azimuth 2.0 (ref. 29), which uses only information about the genome in the region targeted by the sgRNA. We found that among genes identified as common dependencies in either data set, mean sgRNA depletion indeed had a strong relationship to the sgRNA’s Azimuth estimated efficacy (Fig. 4c). Thus, for genes where Azimuth estimates are quite different between data sets, observed phenotype differences are probably due to differences in sgRNA efficacy. For each gene in each library, we calculated the median estimated sgRNA efficacy (MESE) and found cases where differing MESE values appear to explain gene score differences. Some examples of this effect are EIF3F (common essential in Sanger screens with MESE 0.613, non-scoring in Broad screens with MESE 0.398) and MDM2 (strongly selective in Broad screens with MESE 0.585, correlated but not strongly selective in Sanger screens with MESE 0.402) (Fig. 4d).

We next investigated the role of different experimental time points on the screens’ agreement. Given that the Broad used a longer assay length (21 days versus 14 days) we expected differences to be observed between late dependencies across the data sets. Therefore, we compared the distribution of gene scores for genes known to exert a loss of viability effect upon inactivation at an early- or late-time (early or late dependencies)11. While early dependencies have similar score distributions in both data sets (median average score $-0.781$ at the Sanger and $-0.830$ at the Broad), late dependencies are more depleted at the Broad with median average score $-0.402$ compared to $-0.269$ for the Sanger screens (Fig. 5a). The probability of observing a difference at least this extreme for a random set of genes of the same size is $2.57 \times 10^{-78}$.

Many other experimental differences may also contribute to differences in reported response. For example, Lagziel et al. showed that many metabolic gene dependency profiles in Achilles are related to screening media, with e.g. asparagine synthetase (ASNS) notably more dependent in media lacking asparagine. The Broad Institute used provider-recommended media for all Achilles screens, while the Sanger Institute adapted cells to either RPMI or a fifty-percent mix of DMEM and F12. While DMEM lacks asparagine, both RPMI and F12 contain it; thus, ASNS is expected to be a strong dependency only in Broad screens, and only in DMEM or other asparagine-deficient media. We confirmed this result (Fig. 5b). The difference between ASNS dependency in DMEM and either RPMI or DMEM:F12 in Broad screens is significant (Student’s t-test $p = 1.52 \times 10^{-10}$, N = 100 and $p = 0.0173$, N = 80). In contrast, the difference between the RPMI and DMEM:F12 media conditions is not significant in either the Broad ($p = 0.961$, N = 34) or the Sanger ($p = 0.964$, N = 147). Although ASNS is the strongest example, it is likely that some of the differences in other metabolic genes between institutes are explained by media.

Unlike differences in sgRNA efficacy, both time point and media effects are expected to relate to the biological role of late dependencies. As the Broad Institute uses longer screens and includes a greater variety of media, Broad-exclusive dependencies are likely to contain enrichment for gene functional sets. We confirmed this by functionally characterizing, using gene ontology (GO), genes that were exclusively detected as depleted in individual cell lines (at 5% FDR), in one of the two studies, excluding genes with significantly different sgRNA efficacies between libraries. Results showed 29 GO categories significantly
enriched in the Broad-exclusive dependency genes (Broad-exclusive GO terms) for more than 50% of cell lines (Fig. 5c and Supplementary Data 8). The Broad-exclusive enriched GO terms included classes related to mitochondrial and RNA processing gene categories and other gene categories previously characterized as late dependencies. In contrast, no GO terms were significantly enriched in the Sanger-exclusive common dependencies in more than 30% of cell lines.

**Batch effect sources: experimental verification.** To verify that batch effects between the data sets can be removed by changing
**Fig. 4 Influence of reagent library on gene score.** a Distributions of sgRNA depletion score correlations for sgRNAs targeting genes with varying NormLRT scores within each data set (left) and between them (right). Each gene is binned according to the mean of its NormLRT score across the two data sets. The x-axis defines the color gradient. The y-axis reports the average of all correlations between pairs of sgRNAs that belong to the same data set and target that gene. Boxes cover the interquartile range with the median indicated by a horizontal line. Whiskers extend up to 1.5 times the interquartile range with outliers shown as fliers. b Relationship between sgRNA correlation within data sets and gene correlation between data sets. The linear trend is shown for SSD genes. c The mean depletion of guides targeting common dependencies across all replicates with Azimuth estimates of guide efficacy. The x-axis defines the color gradient. d Comparison of Broad and Sanger unprocessed gene scores for genes matching SSD with highest minimum median estimated sgRNA efficacy (MESE) across both libraries (left, TFA2C), common dependency in either data set and greatest difference between KY and Avana MESE (center, EIF3F), and the SSD with worst KY MESE (right, MDM2).

**Fig. 5 Influence of time point.** a Distribution of early and late common dependency gene scores in the Broad and Sanger data sets averaged across cell lines. Boxes cover the interquartile range with the median indicated by a horizontal line. Whiskers extend up to 1.5 times the interquartile range with outliers shown as fliers. b Distribution of corrected gene scores for asparagine synthetase (ASNS) by media and institute. Blue and orange lines indicate the median of nonessential and essential gene scores, respectively. c GO terms significantly enriched in Broad-exclusive dependencies. For each GO term the bar length indicates the ratio of cell lines showing Broad-exclusive dependencies with a statistically significant enrichment of that GO term.
the library and the readout time point, we undertook replication experiments independently at Broad and Sanger institutes, where these factors were systematically permuted. The Broad sequenced cells collected from its original HT-29 and JIMT-1 screens at the 14-day time point and conducted an additional screen of these cell lines using the KY1.1 library with readouts at days 14 and 21. The Sanger used both the Broad’s and the Sanger’s clones of HT-29 to conduct a new KY screen and an Avana screen with readouts at days 14 and 21. Principal component analysis (PCA) of the concatenated unprocessed gene scores, including replication screens, showed a clear institute batch effect dominating the first principal component. By highlighting replication screens, we found that this effect is chiefly due to library choice, with time point playing a smaller role (Fig. 6a, Supplementary Fig. 6a). Changing from Sanger to Broad clones of HT-29 had minimal impact. We examined the change in gene score profile for each screen caused by changing either the library or time point while keeping other conditions constant. Gene score changes induced by either library or time point alterations were consistent across multiple conditions (Fig. 6b). Sanger-exclusive common dependencies were strongly enriched for genes that became more depleted with the KY library, and Broad-exclusive common dependencies were enriched among genes more depleted with the Avana library (Supplementary Fig. 6b). Late dependencies were strongly enriched among genes that became more depleted in the later time points, while early dependencies were not enriched.
(Supplementary Fig. 6c). We compared the deviations in gene score between Broad and Sanger screens under different conditions, first comparing Broad original and replication screens of HT-29 (Fig. 6c) and JIMT-1 (Supplementary Fig. 6d) to the original Sanger screens of the same cell line. Matching library and time point removed most of the average gene score change (batch effect) between institutes, as indicated by the low correlation of the remaining gene score differences in the replication screens with the average gene score change. Specifically, matching Sanger's library and time point reduces the variance of gene scores in HT-29 from 0.0486 to 0.0252 and in JIMT-1 from 0.0556 to 0.0260. We next compared Sanger original and replication screens of HT-29 to the Broad original HT-29 screen. Matching library and time point successfully detrended the data in this case as well; however, the Sanger Avana screens of HT-29 contained considerable excess noise, causing these screens to have a higher overall variance from the Broad than the original screens (0.0486 vs 0.115). Nonetheless, the replication experiments confirm that the majority of batch effects between data sets are driven by the library and time point.

Discussion

Providing sufficient experimental data to adequately sample the diversity of human cancers requires high-throughput screens. However, the benefits of large data sets can only be exploited if the underlying experiments are reliable and robustly reproducible. In this work, we survey the agreement between two large, independent CRISPR-Cas9 knock-out data sets, generated at the Broad and Sanger institutes.

Our findings illustrate a high degree of consistency in estimating gene dependencies between studies at multiple levels of data processing, albeit with the longer duration of the Broad screens leading to stronger dependencies for a number of genes. The data sets are concordant in identifying common dependencies and identifying mean dependency signals. Their agreement is also striking in the more challenging task of identifying which cell lines are dependent on selective dependencies. Indeed, when we compared the two data sets at the level of gene dependency markers we found consistent results at the level of common informative molecular features, as well as with respect to their quantitative strength.

We observed that a source of disagreement across the compared data set is due to diffuse batch effects visible when the whole profiles of individual cell lines are compared. Such effects can be readily corrected with standard methods without compromising data quality, thus making possible integration and future joint analyses of the two compared data sets. Furthermore, much of this batch effect can be decomposed into a combination of two experimental choices: the sgRNA library and the duration of the screen. The effect of each choice on the mean depletion of genes is readily explicable and reproducible, as shown by screens of two lines performed at the Broad using the Sanger’s library and screen duration and a reciprocal screen performed at the Sanger with the Broad library and duration. Consequently, identifying high-efficacy reagents and choosing the appropriate screen duration should be given high priority when designing CRISPR-Cas9 knock-out experiments.

Methods

Unprocessed gene scores. Read counts for the Broad were taken from avana_public_19Q1 (ref. 31) and filtered so that they contained only replicates corresponding to overlapping cell lines and only sgRNAs with one exact match to a gene. Read counts for Sanger were taken from Behan et al.12 and similarly filtered, then both read counts were filtered to contain only sgRNAs matching genes common to all versions of the data. In both cases, reads per million (RPM) were calculated and an additional pseudo-count of 1 added to the RPM. Log fold change was calculated from the reference pDNA. In the case of the Broad, both pDNA and screen results fall into distinct batches, corresponding to evolving PCR strategies. Cell lines sequenced with a given batch were matched to pDNA profiles belonging to the same batch. Multiple pDNA RPM profiles in each batch were median-collapsed to form a single profile of pDNA reads for each batch. Initial gene scores for each replicate were calculated from the median of the sgRNAs targeting that replicate. Each replicate’s initial gene scores for both Broad and Sanger were then shifted and scaled so the median of nonessential genes in each replicate was 0 and the median of essential genes in each cell line was$−1$(ref. 12). Sanger gene scores were taken from the quantile-normalized averaged log fold-change scores, post-correction with CRISPRcleanR32, and globally rescaled by a single factor so that the median of essential genes across all cell lines was$−1$(ref. 12).

Processed gene scores. Broad gene scores were taken from avana_public_19Q1 gene_effect14 and reflect CERES15 processing. The scores were filtered for genes and cell lines shared between institutes and with the unprocessed data, then shifted and scaled so the median of nonessential genes in each cell line was 0 and the median of essential genes in each cell line was$−1$(ref. 12). Sanger gene scores were taken from the quantile-normalized averaged log fold-change scores, post-correction with CRISPRcleanR32, and globally rescaled by a single factor so that the median of essential genes across all cell lines was$−1$(ref. 12).

Batch-corrected gene scores. The unprocessed sgRNA log FCs were mean collapsed by gene and replicate. Data were quantile normalized for each institute separately before processing with ComBat using the R package sva. One batch factor was used in ComBat defined by the institute of origin. The ComBat corrected data were then quantile normalized to give the final batch-corrected data set.

Alternate conditions. Screens with alternate libraries, cell lines, and time points were processed similarly to the Unprocessed data above.

Gene expression data. Gene expression log(Transcript per million +1) data were downloaded for the Broad from the Figrshare repository for the Broad data set. For the Sanger data set, we used fragments per kilobase of transcript per million (FPKM) expression data from Cell Model Passports53. We added a pseudo-count of 1 to the FPKM values and transformed to log. Gene expression values are quantile normalized for each institute separately. For the Sanger data, Ensembl gene ids were converted to Hugo gene symbols using BioMart package in R.

Guide efficacy estimates. On-target guide efficacies for the single-target sgRNAs in each library were estimated using Azimuth 2.0 (ref. 29) against GRCh38.

Comparison of all gene scores. Gene scores from the chosen processing method for both Broad and Sanger were raveled and Pearson correlations calculated between the two data sets. 100,000 gene-cell line pairs were chosen at random and density-plotted against each other using a Gaussian kernel with the width determined by Scott’s rule24. All gene scores for essential genes were similarly plotted in Fig. 1b.

Comparison of gene means. Cell line scores for each gene in both Broad and Sanger data sets with the chosen processing method were collapsed to the mean score, and a Pearson correlation calculated.

Gene ranking, common essential identification. For each gene in the chosen data set, its score rank among all gene scores in its 90th percentile least depleted cell line was calculated. We call this the gene’s 90th percentile ranking. The density of 90th percentile rankings was then estimated using a Gaussian kernel with width 0.1 and the central point of minimum density identified. Genes whose 90th percentile rankings fell below the point of minimum density were classified as common essential.

Identification of selective gene sets. Selective dependency distributions across cell lines are identified using a Likelihood Ratio Test as described in McDonald et al.21. For each gene, the log-likelihood of the fit to a normal distribution and a skew-t distribution is computed using R packages MASS37 and sn36, respectively. In the event that the default fit to the skew-t distribution fails, a two-step fitting process is invoked. This involves keeping the degrees of freedom parameter ($\nu$) fixed during an initial fit and then using the parameter estimates as starting values for a second fit without any fixed values. This process repeats up to 9 times using $\nu$ values in the list (2, 5, 10, 25, 50, 100, 250, 500, 1000) sequentially until a solution is reached. The reported LRT score is calculated as follows:

$$LRT = 2 \times [\ln(\text{likelihood for Skewed}) − \ln(\text{likelihood for Gaussian})]$$

(1)

The numerical optimization methods used for the estimates do not guarantee the maximum of the objective function is reached. In a small number of cases, we failed to find a solution even with multiple attempts. NormLRT scores have been left blank for these genes. Genes with NormLRT scores greater than 100 and mean gene score greater than$−0.5$in at least one institute’s unprocessed data set were classified as SDs.
Binarized agreement of SSDs. For each processing method, Broad and Sanger gene scores were concatenated. Scores for nonessential genes across all cell lines and both institutes were taken as the null distribution, and a left-tailed p-value was calculated for each score. The resulting p-values for each processing method were converted to FDR using the Benjamini–Hochberg algorithm as implemented in the python package statsmodels. The gene score threshold corresponding to a FDR of 0.05 or lower was used to binarize gene scores. These thresholds were −1.02 for unprocessed gene scores, −0.633 for processed gene scores, and −0.765 for corrected gene scores. Cohen’s kappa was calculated for each gene individually. Fisher’s exact test, precision, recall, and AUROC scores were calculated globally for all SSD sensitivities in the three data versions.

Cell line agreement analysis. To obtain the two dimensional visualisations of the combined data set before and after batch correction and considering different gene scores, we computed the sample-wise correlation distance matrix and used this as input into the t-statistic Stochastic Neighbor Embedding (tSNE) procedure, using the t SCE function of the tR package, with 1000 iterations, a perplexity of 100 and other parameters set to their default value.

To evaluate genome-wide cell line agreement we considered a simple nearest-neighbour classifier that, for each dependency profile of a given cell line in one of the two studies, predicted its matching counterpart in the other study. This prediction was based on the correlation distance between one profile and all the other profiles. To estimate the performance of this classifier, we computed a Recall curve for each of the 284 dependency profiles in the tested data set. Each of these curves was assembled by concatenating the number of observed true-positives amongst the first k neighbors of the corresponding dependency profile (for k = 1–293). We then averaged the 294 resulting Recall curves into a single curve and converted it to percentages by multiplying by 100/294. Finally, we computed the area under the resulting curve and normalized it by dividing by 293. We considered the area under this curve (nAUC) as a performance indicator of the k-nearest neighbor.

Cell line profiles agreement in relation to data quality. First, to estimate the initial data quality we calculated true positive rates (TPRs, or Recalls) for the sets of significant dependency genes detected across cell lines, within the two studies. To this aim, we used as positive control a reference set of a priori known essential genes. For the agreement assessment via ROC indicators (Recall, Precision and Specificity), for each of the two studies in turn we picked the most significant 20 clones, two versions exist, the original and an alternative that was eventually grown of these screens, we subtracted the gene scores of the version performed with the Avana library to create library differences, we took all screens that had been duplicated in each library with all other conditions (time point, clone, and screen location) kept constant. For each of these screens, we subtracted the gene scores of the version performed with the KY library from the version performed with the Avana library to create library differences. For the case of Sanger’s day-14 KY screen of the Sanger HT-29 clone, two versions exist, the original and an alternative that was eventually grown on the Broad HT-29 screen. We used the median difference. For each cell line if the corrected p-value resulting from the corresponding test was < 0.05.

Principal component analysis of the batch effect. The Broad and Sanger unprocessed gene scores and the gene scores for the alternate conditions tested by both institutes were concatenated into a single matrix with a column for each screen. Principal components were found for the transpose of this matrix, where each row is a screen and each column a pseudogene. Components 1 and 2 were plotted for all original screens and the alternate screens for each HT-29 (Fig. 6a) or JIMT-1 (Supplementary Fig. 6a). The aspect ratio for the plot was set to match the relative variance explained by the first two principal components.

Consistency of time point and library effects on gene scores. To evaluate library differences, we took all screens that had been duplicated in each library with all other conditions (time point, clone, and screen location) kept constant. For each of these screens, we subtracted the gene scores of the version performed with the KY library from the version performed with the Avana library to create library difference profiles. For the case of Sanger’s day-14 KY screen of the Sanger HT-29 clone, two versions exist, the original and an alternative that was eventually grown on the Broad HT-29 screen: the original (Avana library at day 21), then with the Avana library if the corrected p-value resulting from the corresponding test was < 0.05.
library at day 14, the KY library at day 21, and the KY library at day 14, generating four arrays indexed by gene which form the y-axes in the succession of plots in Fig. 6c. We also computed the mean score of each gene across all original Broad screens and subtracted it from the mean score of each gene across all the original Sanger screens to form the x-axis of all four plots. For each condition, the standard deviation of the HT-29 screen differences (y-axes) was computed along with the correlation of the HT-29 screen differences with the mean differences (x-axis). The plots themselves are Gaussian kernel density estimates. We repeated this process for JIMT-1 (Supplementary Fig. 6d) and then for HT-29 while swapping the roles of Broad and Sanger (Fig. 6d). For the Sanger alternate condition screens we used the Sanger clone of HT-29, and for its day 14 KY screen we used the Sanger’s original HT-29 screen.

Replication experiments. The replication screens at Broad and Sanger were performed using the normal current protocol of the respective institution except with respect to the specifically noted changes to the library (and the associated primer sequences required for post-screen amplification of the sgRNA barcodes) and the time point. See Supplementary Methods for details.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The data used for this paper have been posted to Figshare (https://doi.org/10.6084/m9.figshare.7970993.v1).

Code availability
Scripts to perform all analyses and generate figures are available at https://github.com/DepMap-Analytics/Comparative-Analysis.

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Author contributions
J.M.D, F.I. and A.T. conceived and designed the study. J.M.D. and C.P. conducted the analyses described under Results. J.M.D., C.P. and F.I. wrote the paper and produced the figures. J.T. wrote the paper, H.N. produced figures and curated data. J.M.D. munged and collated gene scores. C.P. munged and collated cell characterizations. J.K.-B. produced the script used to calculate NormLRTE scores. V.Z., S.P., S.T.Y. and D.E.R. conducted the Broad’s replication of Sanger screens, while F.B., R.S. and C.M.B conducted Sanger’s replications and curated corresponding data. J.G.D and K.Y. provided ideas and contributed ideas on some of the analyses. J.S.B., T.R.G., W.C.H and M.J.G. edited the paper and contributed ideas on some of the analyses. J.S.B., T.R.G., W.C.H and M.I.G. acquired funds and contributed to study supervision. A.T. and F.I. acquired funds and supervised the study.

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**Additional information**

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