Identifying drug-gene interactions from CRISPR knockout screens with drugZ

Gang Wang[1], Michal Zimmermann[2], Keith Mascall[3], W. Frank Lenoir[1,4], Jason Moffat[5,6], Stephane Angers[3], Daniel Durocher[2,6], Traver Hart[1]*

1 - Department of Bioinformatics and Computational Biology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

2-Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, 600 University Avenue, Toronto, ON, M5G 1X5, Canada

3-Department of Pharmaceutical Sciences, Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada

4-UTHealth Graduate School of Biomedical Sciences, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

5-Donnelly Centre, University of Toronto, Toronto, ON, Canada

6-Department of Molecular Genetics, University of Toronto, Toronto, ON, M5S 3E1, Canada

*To whom correspondence should be addressed: traver@hart-lab.org

Abstract

Chemogenetic profiling enables the identification of gene mutations that enhance or suppress the activity of small molecules. This knowledge provides insights into drug mechanism-of-action, genetic vulnerabilities, and resistance mechanisms, all of which may help stratify patient populations. We present drugZ, an algorithm for identifying both synergistic and suppressor chemogenetic interactions from highly sensitive CRISPR screens, available at github.com/hart-lab/drugz. In screens for interactions with a poly(ADP-ribose) polymerase (PARP) inhibitor, DrugZ identifies a greater fraction of the homologous recombination repair pathway than contemporary methods, and confirms KEAP1 loss as a resistance factor for ERK inhibitors.

Introduction

The ability to systematically interrogate multiple genetic backgrounds with small molecule "perturbagens" is known as chemogenetic profiling. While this approach has many applications in chemical biology, it is particularly relevant to cancer therapy, where clinical compounds or small molecule probes or are profiled to identify mutations that inform on genetic vulnerabilities or resistance mechanisms [1]. Systematic surveys of the fitness effects of environmental perturbagens across the yeast deletion collection [2] offered insight into gene function at a large scale, while profiling of drug sensitivity in heterozygous deletion strains identified genetic backgrounds that give rise to increased drug sensitivity [3]. Now, with the advent of CRISPR technology and its adaptation to pooled library screens in mammalian cells, high resolution chemogenetic screens can be carried out directly in human cell cells [4-6]. Major advantages to this approach include the ability to probe all human genes, not just orthologs of model organisms; the analysis of how drug-gene interactions vary across different tissue types, genetic backgrounds, and epigenetic

states; and the identification of suppressor as well as synergistic interactions, that may indicate possible mechanisms of acquired resistance or pre-existing sources of resistant cells in heterogeneous tumor populations.

Design and analysis of CRISPR-mediated chemogenetic interaction screens in human cells can be problematic. Positive selection screens identifying genes conferring resistance to cellular perturbations typically have a high signal-to-noise ratio, as only mutants in resistance genes survive[5, 7-11]. On the other hand, negative selection CRISPR screens require growing perturbed cells over 10 or more doublings to allow sensitive detection of genes, whose knockout leads to moderate fitness defects. Adding a drug interaction necessitates dosing at low levels (~IC20) to balance between maintaining cell viability over a long timecourse and inducing drug-gene interactions beyond native drug effects.

Several algorithms currently exist for the analysis of drug-gene interaction experiments [12, 13]. Most rely on adapting methods originally developed for the analysis of RNAseq differential expression data, which is typically characterized by relatively high read counts across genes. High read counts enable the statistically robust detection and ranking of differential expression of genes (in RNA-seq) or abundance of guide (g) RNA (in CRISPR screens) using approaches such as the negative binomial P-value model, a trend explored thoroughly in [13]. However, low read counts per gRNA are common in CRISPR data, and are a fundamental feature of genes with fitness defects, leading to a severe loss of sensitivity when applied to CRISPR screens for synthetic chemogenetic interactions.

In this study, we describe drugZ, an algorithm for the analysis of CRISPR-mediated chemogenetic interaction screens. We apply the algorithm to identify genes that drive normal cellular resistance to the clinical PARP inhibitor olaparib in three cell lines. We demonstrate the greatly enhanced sensitivity of drugZ over contemporary algorithms by showing how it identifies more hits with higher enrichment for the expected DNA damage response pathway, and further how it identifies both

synergistic and suppressor interactions. We provide all software and data necessary to replicate the analyses presented here.

Methods

We created the drugZ algorithm to fill a need for a method to identify chemogenetic interactions in CRISPR knockout screens. In a pooled library CRISPR screen, the relative abundance of each gRNA in the pool is usually sampled immediately after infection and selection. To identify genes whose knockout results in a fitness defect ("essential genes"), the cells are grown for several doublings and the relative abundance of gRNA is again sampled by deep sequencing of a PCR product amplified from genomic DNA. The relative frequency of each gRNA is compared to the initial population and genes whose targeting gRNA show consistent dropout are considered essential genes.

In a chemogenetic interaction screen, the readout of choice is somewhat different. The relative abundance of gRNA in a treated population is compared to the relative abundance of an untreated population at the same timepoint (Fig 1a). In this context, an experimental design with paired samples is particularly powerful, as it removes a major source of variability across replicates.

We calculate the $\log_2$ fold change of each gRNA in the pool by normalizing the total read count of each sample at the same timepoint and taking the log ratio, after adding a pseudocount of 5 reads to each gRNA. We find a pseudocount of 5 to be a good compromise between minimizing low-readcount artifacts and suppressing true biological signal, though we leave this as a user-modifiable parameter in the software. We then calculate the mean and standard deviation of all gRNA targeting a set of negative control genes – the reference nonessential gene set (NEG) in [14, 15] – and use these values to determine a Z score for each gRNA. The guide Z score of all gRNA across all replicates is summed to get a gene-level sumZ score, which is then normalized (by dividing by the square root of the number of summed terms) to the

final normZ. A P-value is calculated from the normZ, and false discovery rates are determined using the method of Benjamini and Hochberg [16]. The analytical workflow is depicted in Figure 1b. The open-source Python software can be downloaded from github.com/hart-lab/drugz.

Results and Discussion

To benchmark the method, we evaluated screens to identify modifiers of the response to the PARP inhibitor olaparib in three cell lines, RPE1-hTERT, HeLa, and SUM149PT (Zimmermann et al., submitted). The screens were performed using the TKOv1 library of 90k gRNA targeting 17,000 genes [17]. After infection and selection, each cell line was split into 3 replicates, passaged at least once, and each replicate was further split into control and olaparib-treated populations, providing a paired-sample experimental design (Figure 1a).

We calculated normZ scores, P-values, and false discovery rates using drugZ in SUM149PT cells. We also analyzed the same data with four contemporary methods, STARS [7], MAGeCK [13], edgeR [18], and RIGER [19]. We were surprised to note that while drugZ produced an increasing number of hits as FDR thresholds were relaxed, the other methods produced substantially lower numbers of hits at the same FDR thresholds (Figure 1c). We tested each statistical model's overall goodness of fit by confirming the uniform distribution of null P-values (Figure 1d) and further observed the absence of a spike of "hits" at low P-values for the other methods.

Having a large number of hits does not necessarily mean that the hits are meaningful. We evaluated the quality of the hits returned by each method by measuring the functional coherence of each gene set. The PARP inhibitor olaparib was developed specifically to exploit the observed synthetic lethal relationship between PARP1 and the BRCA1/BRCA2 genes [20, 21]. Subsequent studies have shown it to be effective against a general deficiency in homologous recombination

repair, known as HRD [22]. We therefore calculated the enrichment of each hit set for genes in the DNA damage response (DDR) pathway as annotated in the Reactome database [23]. Hits found with drugZ show strong enrichment for DDR genes across a range of FDR thresholds (Figure 1e), while the other methods show consistently lower enrichment.

Extreme P-values can be achieved when sample sizes are large, even if the effect size is moderate, and DrugZ returned a larger number of hits at each FDR threshold. To confirm that the P-value difference was not merely a function of sample size, we further looked at the number of DDR-annotated genes in each hit set. Figure 1e shows that roughly twice as many DDR-annotated genes were discovered using DrugZ; moreover, the enrichment of DDR genes among total hits is also consistently greater with DrugZ than with other methods (Figure 1f). We repeated these analyses in HeLa cervical cancer cells and hTERT-immortalized RPE1 epithelial cells, and found that drugZ generally finds more hits with a higher proportion of DDR genes (Supplementary Figure 1), resulting in greater enrichment for annotated genes (Figure 1g-h). The combination of larger sets of hits and greater enrichment for expected results clearly indicates that drugZ accurately and sensitively identifies chemogenetic interactions.

The drugZ algorithm can also be used to identify suppressor interactions; that is, genes whose perturbation reduces drug efficacy. While *BRCA1* mutation is synthetic lethal with *PARP1*, subsequent mutation of *TP53BP1* is associated with acquired resistance to the PARP inhibitor [24]. Drug-gene interactions resulting in positive Z-scores reflect such suppressor interactions. Indeed, *TP53BP1* is the 11th-ranked suppressor interaction in *BRCA1*-deficient SUM149PT cells, with a normZ score of 3.98 (FDR 0.05).

The decreased sensitivity of competing methods to identify chemogenetic interactions under these experimental conditions is often the result the biology of the system under investigation, undermining the applicability of the statistical

approach being used. In two of the methods (MAGeCK and edgeR), the negative binomial model is used to determine a P-value for the differential abundance of each gRNA. Under this model, the P-value is strongly influenced by the number of reads in each sample with low read counts.

Extensive studies in yeast indicate that genes with moderate fitness defects have synergistic genetic interactions with more partners than genes without any appreciable fitness phenotype [25, 26]. In CRISPR screens in mammalian cell lines, moderate fitness defects manifest as lower gRNA read counts at later timepoints. Thus, to discriminate the effects of genetic and chemogenetic interactions on these genes, a method which does not penalize low-readcount observations must be employed. The three methods, which accept low-count observations (drugZ, STARS, and RIGER; Supplementary Figure 2) consistently outperform the two methods that don't (Figure 1c-h, Supplementary Figure 1).

To ensure that the drugZ algorithm is generalizable, we applied it to a separate set of chemogenetic interaction screens in pancreatic cancer cell lines using the ERK1/2 inhibitor SCH772984. Oncogenic mutations in KRAS drive constitutive signaling in the MAP kinase pathway and are associated with proliferation and survival signals. Consistent with current models of RAS pathway activation, knockout of inhibitor target *MAPK1* and its downstream target *RPS6KA3* have strong synthetic interactions with ERK inhibitor in two of the cell lines (FDR < 0.01; Figure 2). In a third cell line, HPAF-II, the top synthetic interactor was drug transporter *ABCG2*. Activity of this drug resistance gene may account for this cell line's resistance to ERK inhibition and the lack of other synthetic effectors in this screen. Epoxide hydrolase *EPHX2* and ubiquitin ligase adapter *KEAP1* are the top two suppressors of ERK inhibitor activity in three cell lines, suggesting these genes are required for normal function of the inhibitor. KEAP1 loss of function was identified as a modulator of MAP kinase pathway inhibitors in a panel of positive selection screens in multiple cell lines[11], but EPHX2 is a novel candidate resistance gene and may be specific to MAPK1 inhibition, to the pancreatic cancer cell lines screened, or simply to the

metabolism of the specific inhibitor used. Notably, the ERK inhibitor screens yielded individual synthetic and suppressor hits, in contrast with the PARP inhibitor screens, which showed broad interaction across the HR pathway, confirming the general applicability of drugZ in detecting drug-gene interactions.

Conclusions

Identifying the genetic drivers of drug effectiveness and resistance is critical to realize the promise of personalized medicine. Chemogenetic interaction screens in mammalian cells using CRISPR knockout libraries have so far been primarily used in a positive selection format to identify the genes, pathways and mechanisms of acquired resistance to chemotherapeutic drugs. However, negative selection screens to identify the underlying architecture of drug-gene interactions have been difficult to carry out and to analyze in large part due to the lack of robust analytical tools.

We describe the drugZ algorithm, which calculates a moderated Z-score for pooled library CRISPR drug-gene interaction screens. By exploiting a paired-sample experimental design and by taking into account the moderate single mutant fitness defects associated with many genes involved in drug-gene interactions, the drugZ algorithm offers significantly improved sensitivity over contemporary analysis platforms. We demonstrate the validity of our hits by showing the strong enrichment for genes involved in the DNA damage response in a screen for interactions with the PARP inhibitor olaparib and the precise detection of MAPK pathway effectors in an ERK inhibitor screen.

We further show that both synergistic and suppressor interactions can be identified in the same screen, as previously identified PARP resistance gene *TP53BP1* is a suppressor hit in *BRCA1*-mutant SUM149PT cells. Moreover, both synthetic target *MAPK1* and suppressor genes *EPHX2* and *KEAP1* are identified in ERK inhibition screens. *KEAP1* deletion or mutation is frequently found in KRAS-driven lung

adenocarcenomas and may present an obstacle to ERK inhibitor therapy in these tumors.

With the ability to perform saturating genetic perturbation screens across a wide range of cells, CRISPR pooled library screens are transforming mammalian functional genetics and holds the promise of identifying new cancer drug targets. Chemogenetic interaction screens extend this capability by enabling the systematic surveying of the landscape of drug-gene interactions across cancer-relevant genetic backgrounds. Understanding this variation may lead to more precise therapies for patients as well as the development of synergistic drug combinations for genotype-specific treatments.

Acknowledgments

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Figures

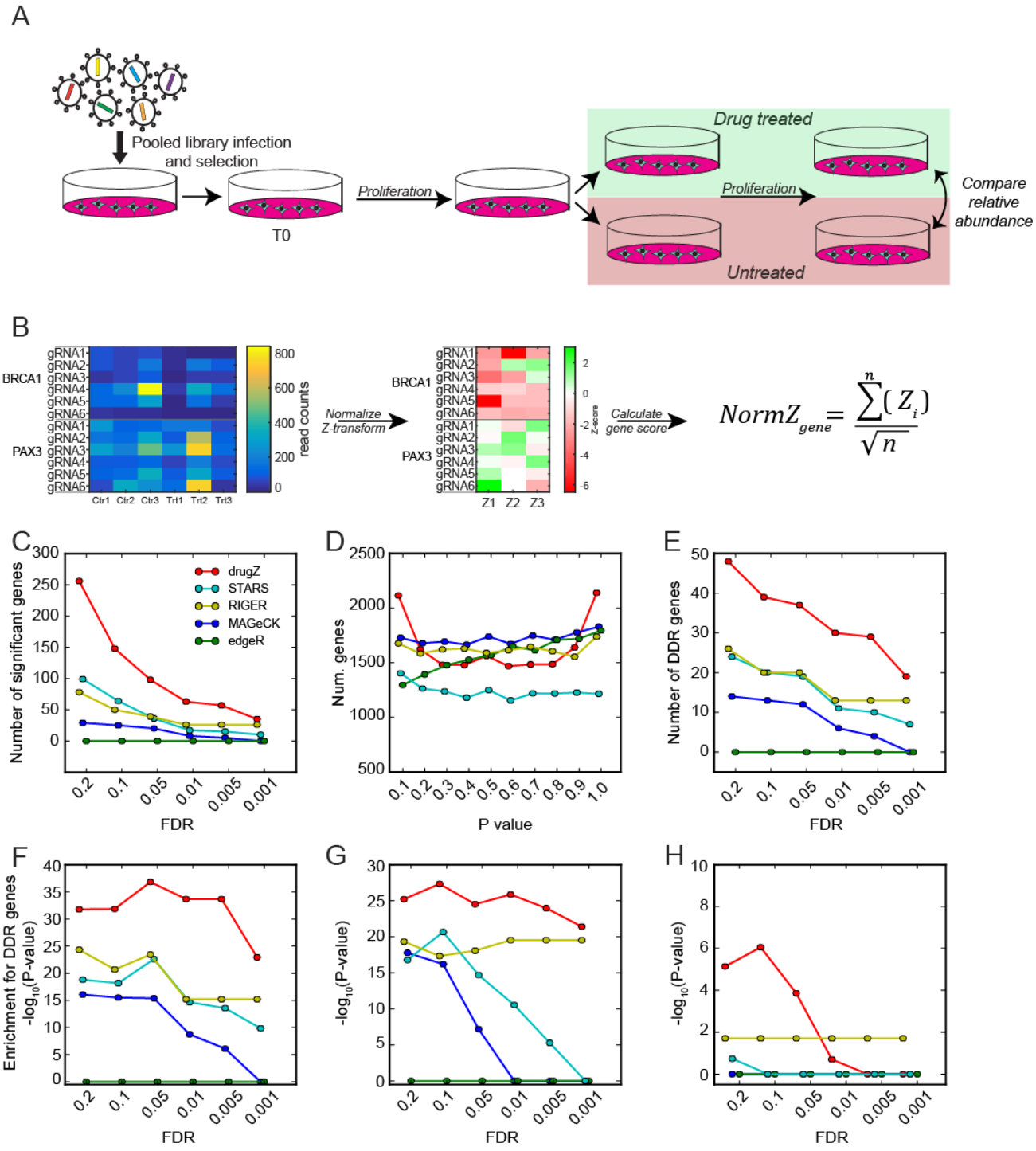


Figure 1. Workflow. (A) In a drug-gene interaction screen, a population of cells is infected with a pooled library of virus-encoded CRISPR guide RNAs. After selection, proliferating cells are split into drug treated and untreated control samples. After

several doublings, genomic DNA is collected and the relative abundance of CRISPR gRNA sequences in the treated and control populations is compared. (B) DrugZ processing steps include normalizing read counts, calculating the log2 fold change of treated samples relative to control, transforming to a Z-score based on the distribution of fold changes of a defined set of negative controls, and collapsing guide-level Z-scores into a gene-level Z-score. (C) In a screen of SUM149PT cells +/- PARP inhibitor olaparib, the number of hits called by each method is plotted vs. FDR threshold, as reported by each software package. (D) Distribution of P-values across all genes is uniform for most methods. (E) Number of annotated DNA damage response genes in hits reported in SUM149PT olaparib screen, plotted vs. FDR threshold. (F) Enrichment (hypergeometric test) for DDR genes in SUM149PT screen. (G) Enrichment for DDR genes in HeLa olaparib screen. (H) Enrichment for DDR genes in RPE1 olaparib screen.

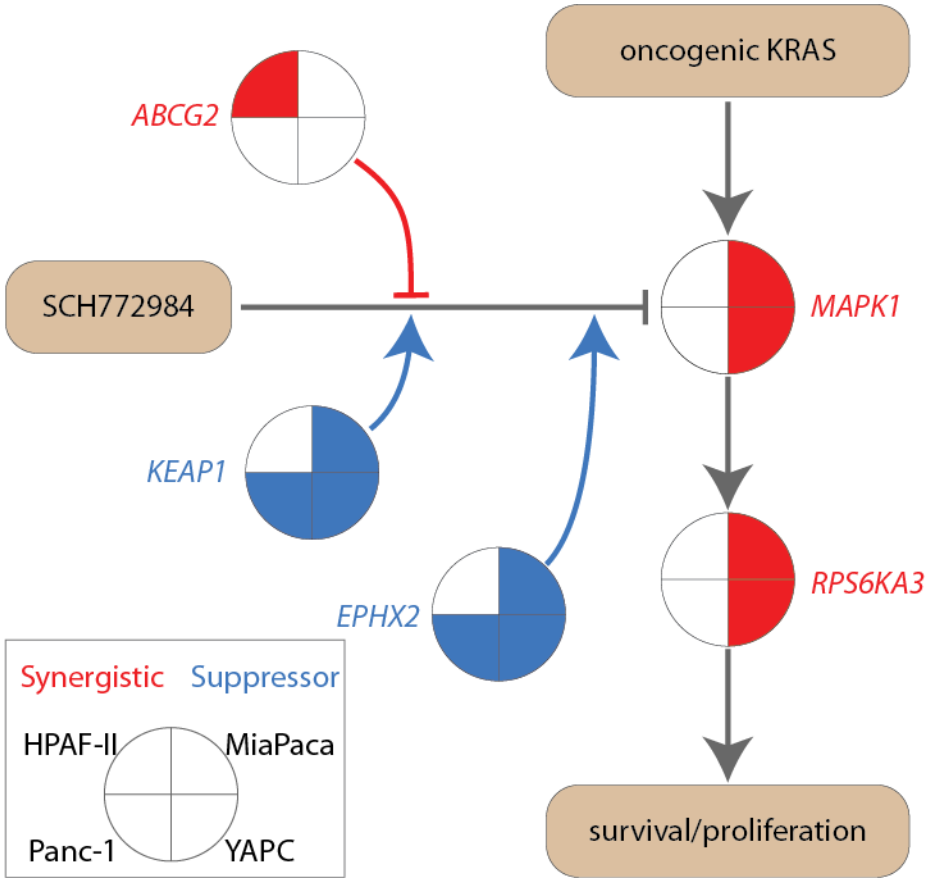
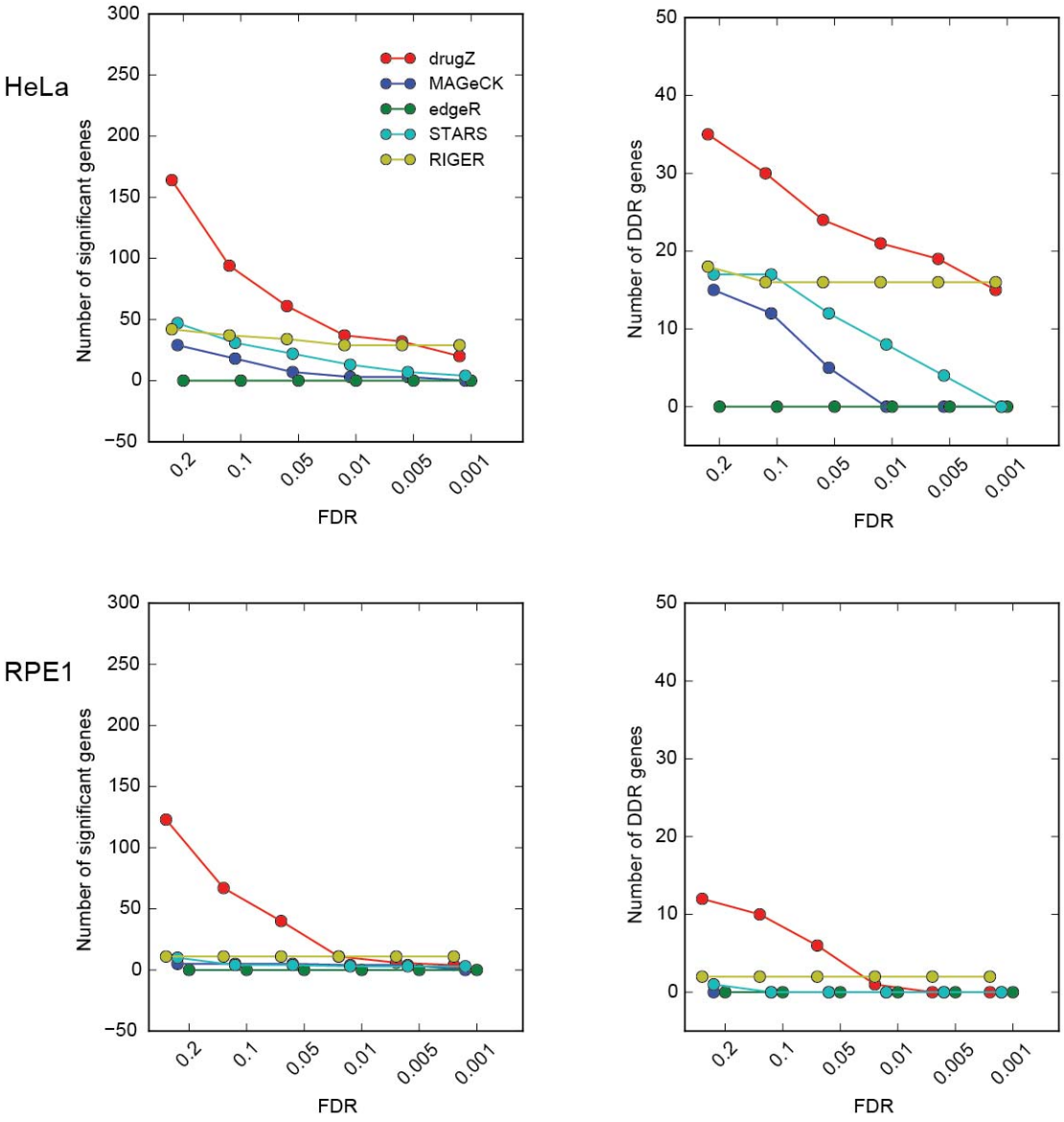
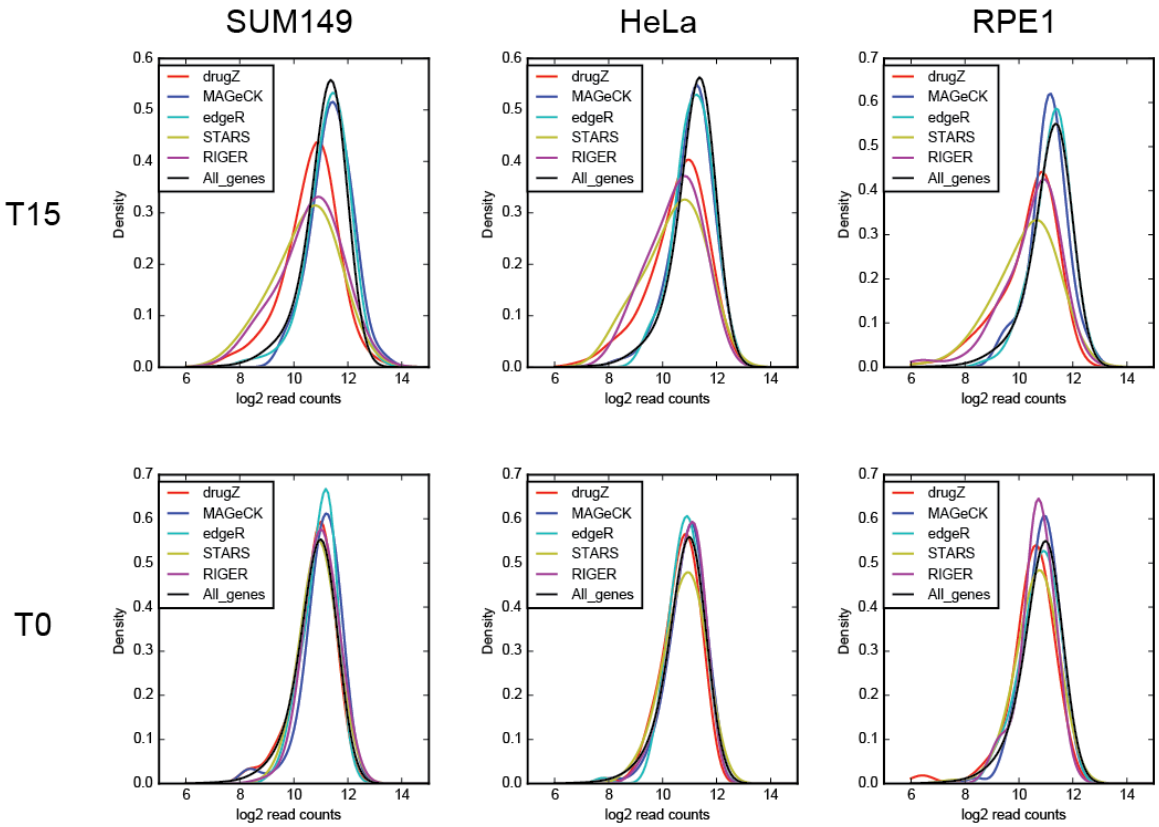


Figure 2. Synergistic and suppressor interactions in ERK inhibitor screen in four pancreatic cancer cell lines. Oncogenic *KRAS* induces proliferation signal through the MAP kinase pathway. SCH772984 target *MAPK1* and effector gene *RPS6KA3* are synthetic lethal in two cell lines, and multidrug transporter *ABCG2* is synthetic lethal in a third. *KEAP1* and *EPHX2* knockout suppresses inhibitor activity in three cell lines.

Supplementary Figures



Supplementary Figure 1. Olaparib screens in other cells. Total hits (left) and annotated DDR genes (right) plotted against FDR threshold for the five methods compared here. Top panels are HeLa cells, bottom are RPE1-hTERT.



Supplementary Figure 2. Relative abundance of hits by each method. The distribution of read counts for all genes in the top 5% of hits by each method at the endpoint of the screen (TOP). Three methods (drugZ, STARS, RIGER) detect hits at low read count, and sensitively detect drug interactions with genes that have knockout fitness defects. (Bottom) Hits show no bias in abundance at the beginning of the screen.