Forward Genetic Screening and Single Cell Analysis with CRISPRi Transcriptional Repressors

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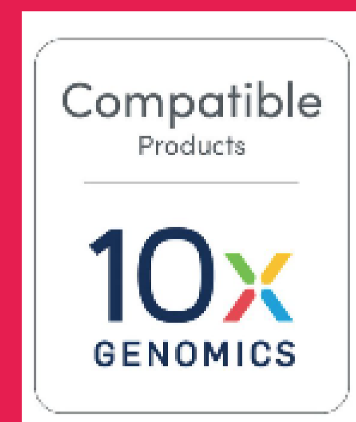
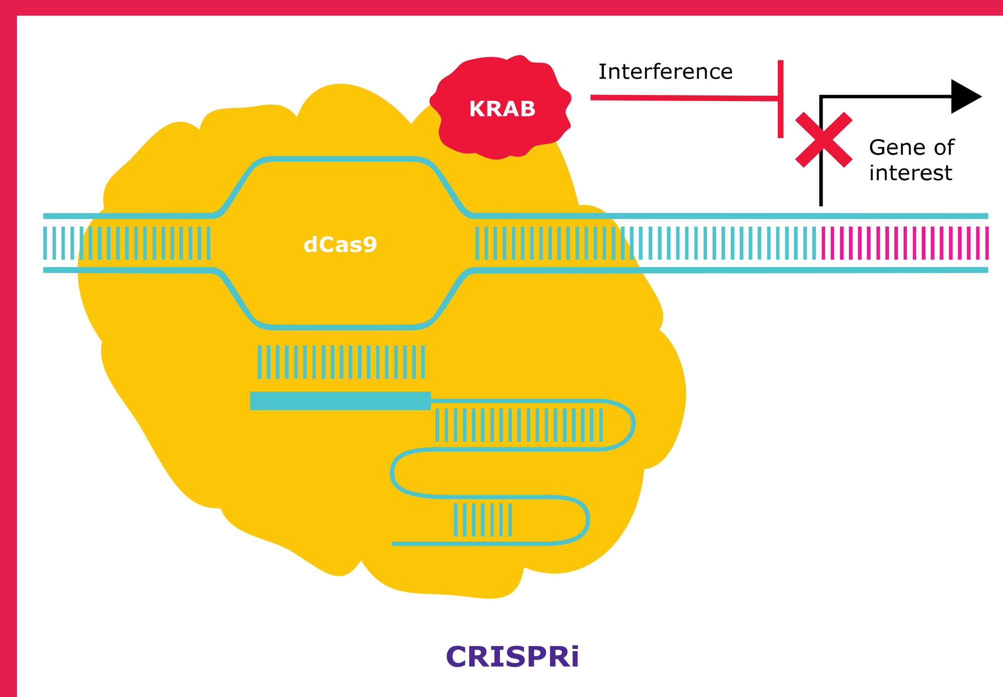
We optimized CRISPRi for superior knockdown.

Focused and whole-genome library screens paired with single-cell analysis drive discovery of novel interactions.





Introduction and Relevance

The power of CRISPR for genome engineering, coupled with the ability to perform large-scale, whole genome, loss-of-function screening has allowed for new breakthroughs identifying gene pathways in drug resistance and disease. CRISPR is most commonly used to create double-stranded breaks that often result in loss of gene function (CRISPR-KO). However, the full extent of CRISPR's utility extends beyond just targeted cutting of DNA. Nuclease-independent applications of CRISPR provide all the targeting specificity but for delivery of cargo, such as effector domains for activation (CRISPRa) or repression (CRISPRi) of target gene expression.

CRISPRi allows for targeted inhibition of gene function by delivering transcriptional repressor domains to a specific target sequence using modified dCas9+gRNA complexes. Gene knockdown is complementary to CRISPR and functional genomic screening tools. We have developed an optimized CRISPRi system using sequence modifications and guide design strategies that maximize knockdown efficiency. We have also developed 10x Genomics-compatible vectors for single-cell RNAseq. **CRISPRi contributes to a powerful toolbox for stand-alone and orthogonal approaches to discovery and detailed functional genomic analysis at different scales.**

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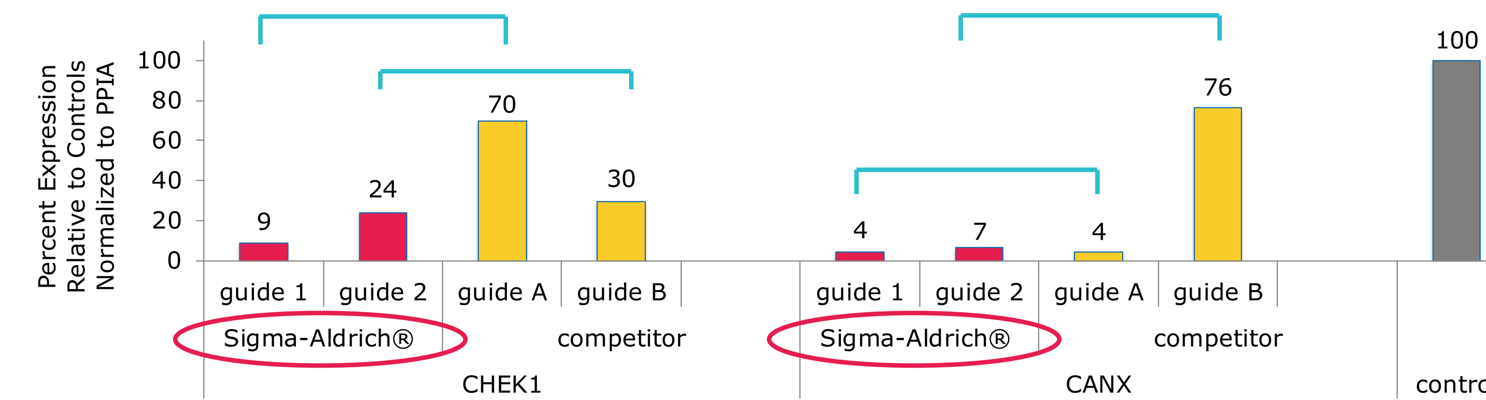
CRISPRi Optimization

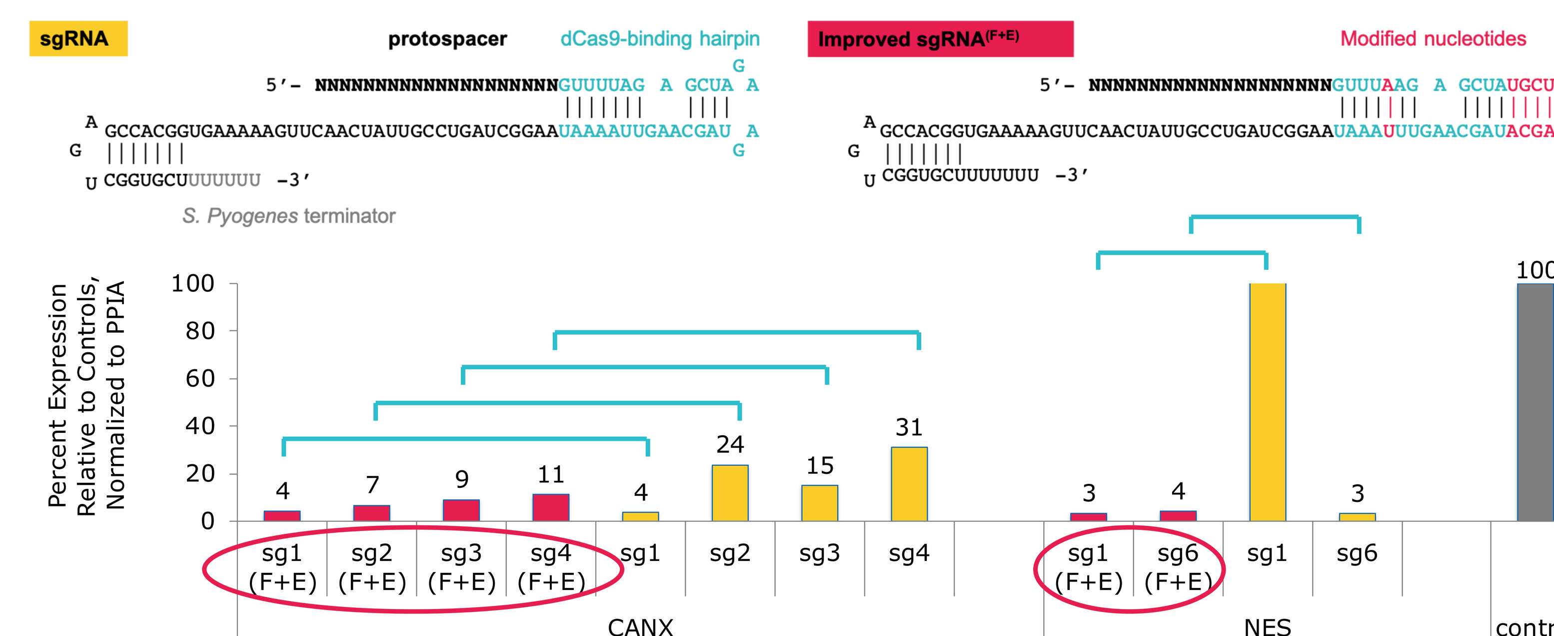
Sigma-Aldrich® CRISPRi guide design enhances guide function prediction

We compared multiple guide design algorithms against the top two guides for targets identified as "hard to repress." Our algorithm incorporates nucleosome positioning data and machine learning to better predict guide function.



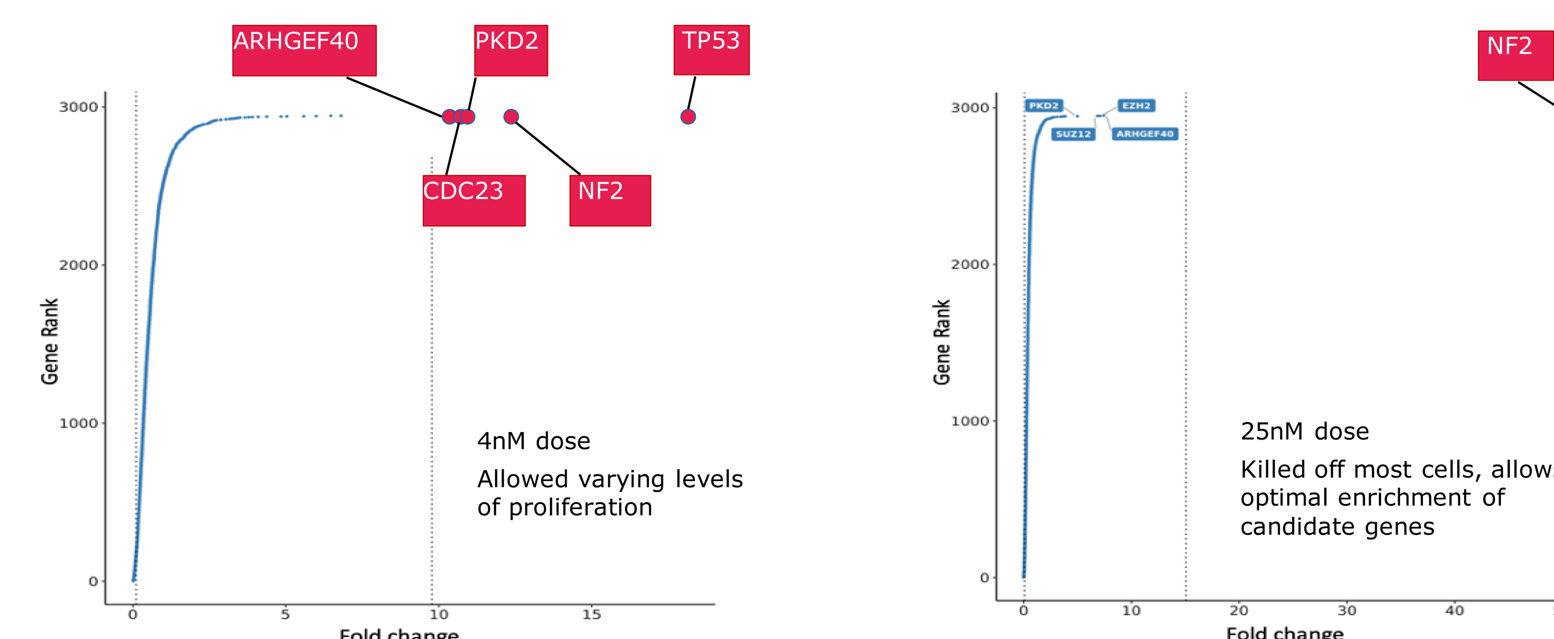
Improved guide RNA scaffold increases efficiency and consistency of knockdown

Previous studies have shown that sequence modifications to the guide scaffold improve transcription and stability of the sgRNA (Chen et al., 2013 *Cell*). Two A-U nucleotides in the DNA-binding hairpin were flipped to eliminate a putative RNA Pol III terminator. Extending the length of the stem loop further enhanced stability in cells. Incorporation of these sequence modifications (F+E) in sgRNAs improves CRISPRi function.



Genome-Scale Screening With CRISPRi

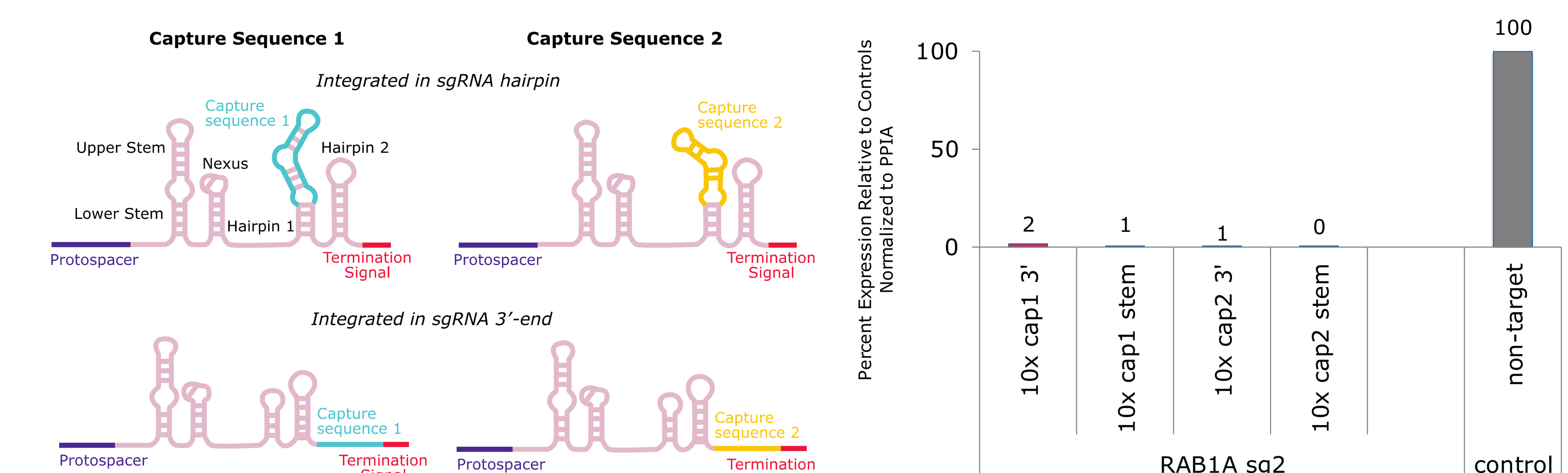
We performed an enrichment screen to implicate new genes and pathways responsible for resistance to Paclitaxel (PAX)-mediated cell death in human lung adenocarcinoma (A549) cells. Cells stably expressing KRAB-dCas9 were transduced with the Sigma-Aldrich® CRISPRi Cancer and Apoptosis subpool of lenti guide RNAs. While our analysis (MAGeCKFlute) identified anticipated genes (e.g., TP53), we also identified a novel role for NF2/merlin in PAX-mediated cell death. This data shows the utility of CRISPRi screening to identify novel genes in and pathways involved in drug and disease phenotypes.



Single-Cell Analysis

Development of 10x Genomics-compatible vectors for single-cell analysis of CRISPRi

Due to high efficiency and universal guide design rules, CRISPRi is an excellent platform for whole-genome screening. To facilitate deeper functional genomics dives, we developed 10x Genomics-compatible reagents to pair single-cell RNASeq with CRISPRi. This strategy is well-suited for conducting in-depth phenotypic analysis on focused guide pools or on top hits from larger screens. Capture sequences are incorporated into the guide RNA scaffold and do not alter knockdown efficiency.



Summary and Significance

We developed **whole-genome and focused CRISPRi libraries** and vectors suitable for analyzing the effects of targeted gene knockdown on cells to discover genes and pathways responsible for drug and disease phenotypes.

We incorporated several different **CRISPR improvements to optimize and enhance knockdown efficiency** and have shown superior results across multiple targets and cell types.

Our **10x Genomics-compatible CRISPR vectors** result in efficient knockdown and allow for robust capture and analysis at the single-cell level.

These technologies combined offer a powerful functional genomics toolbox for the discovery and dissection of genes and pathways with whole-genome to single-cell resolution.

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