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Data Sheet

CRISPRi Whole Genome Libraries

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-KO and CRISPRa, and has distinct advantages over existing loss-of-function strategies like RNAi.

Although these systems have evolved in recent years to accommodate numerous modifications, relatively few advancements have been made to eukaryotic CRISPRi technologies. However, in **Figure 1** we outline one such enhancement.

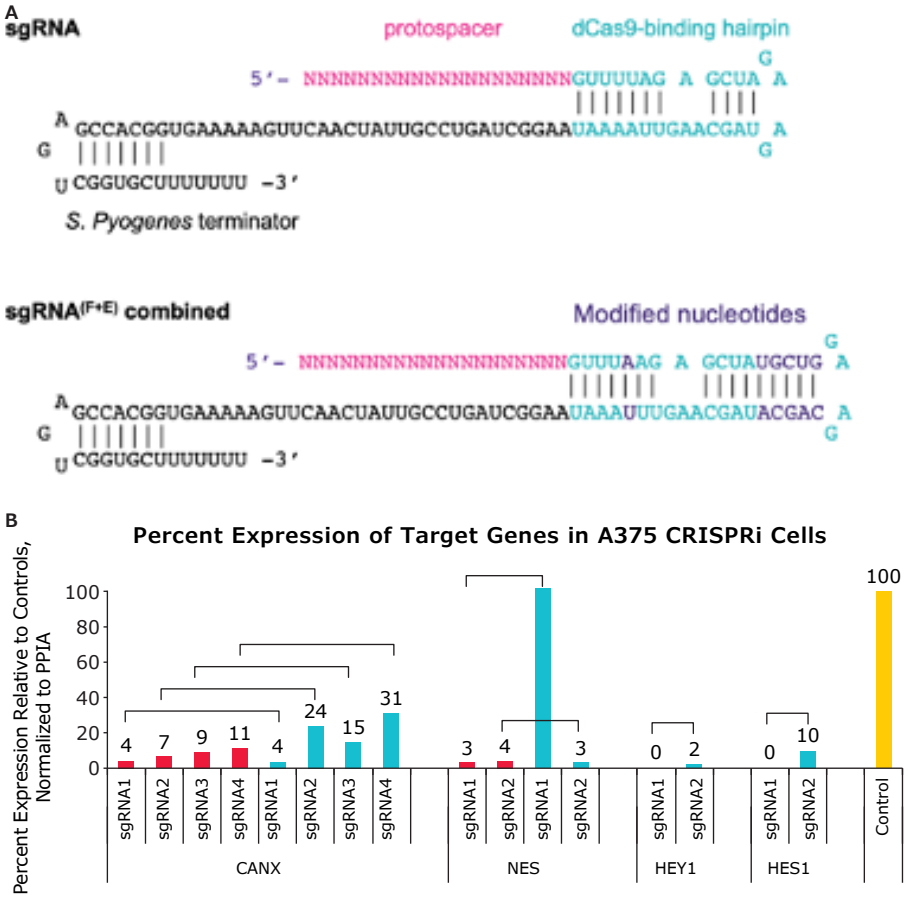


Figure 1. Scaffold modifications and effects on gene knockdown (A) Diagram showing improvements to the CRISPRi sgRNA scaffold. (Adapted from Chen et al., 2013 Cell)[1]. Nucleotides in purple were modified including an A-U flip and extending of the stem loop. **(B)** Graph shows relative expression levels of target genes in stable KRAB-dCas9 cells as determined using qRT-PCR, comparing different guide scaffolds. Bars in cyan represent sgRNAs without modified scaffolding; bars in red represent sgRNAs of identical sequences containing the modification and showing significant improvement in knockdown efficiency. HEY1 and HES1 represent easy-to-repress targets. CANX represents a difficult-to-repress target. NES previously showed high levels of inconsistency across guides.

Comparison of Systems

To further ensure the most efficient knockdown possible, we tested alterations to the CRISPRi system, reported in the literature. Scientists performed a series of experiments comparing Krüppel-associated box (KRAB) to MeCP2 as the primary transcriptional repressor, results are reported in **Figure 2**. We also compared knockdown efficiency in top-ranked guides from different algorithms, results are reported in **Figure 3**.

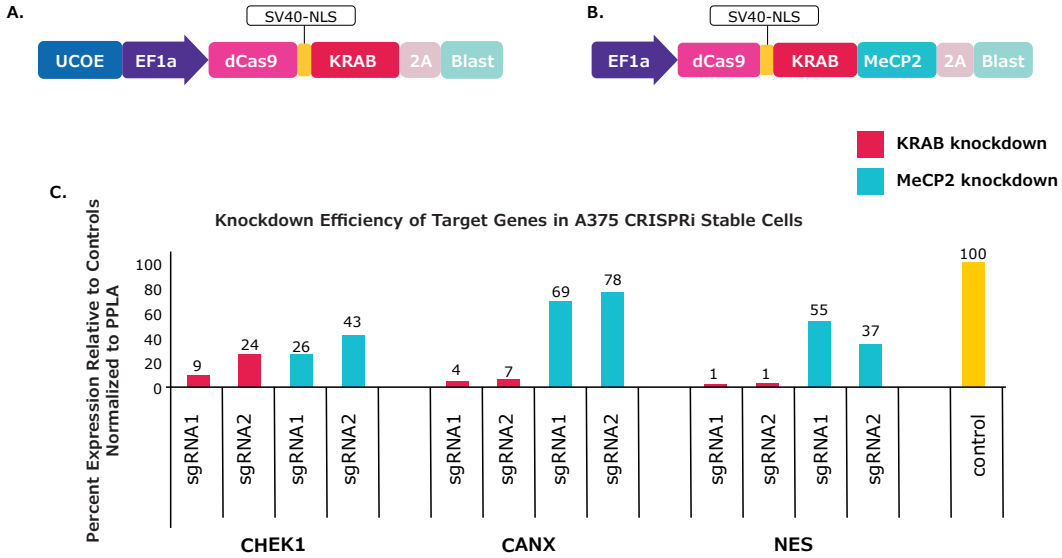


Figure 2. Sigma-Aldrich® KRAB-dCas9 drives superior targeted knockdown efficiency. (A) Diagram of construct developed by Sigma-Aldrich®. **(B)** Diagrams of constructs adapted from Yeo et al.[2] with an additional MeCP2 domain added to the KRAB domain. **(C)** Graph shows relative expression levels of target genes in stable CRISPRi cells as determined using qRT-PCR. The Sigma-Aldrich® system shows superior knockdown efficiency when compared to the MeCP2 system in all targets tested. Demonstrated in CHEK1 and CANX, known difficult-to-repress targets as well as a target that knocks down more efficiently in this cell line, NES.

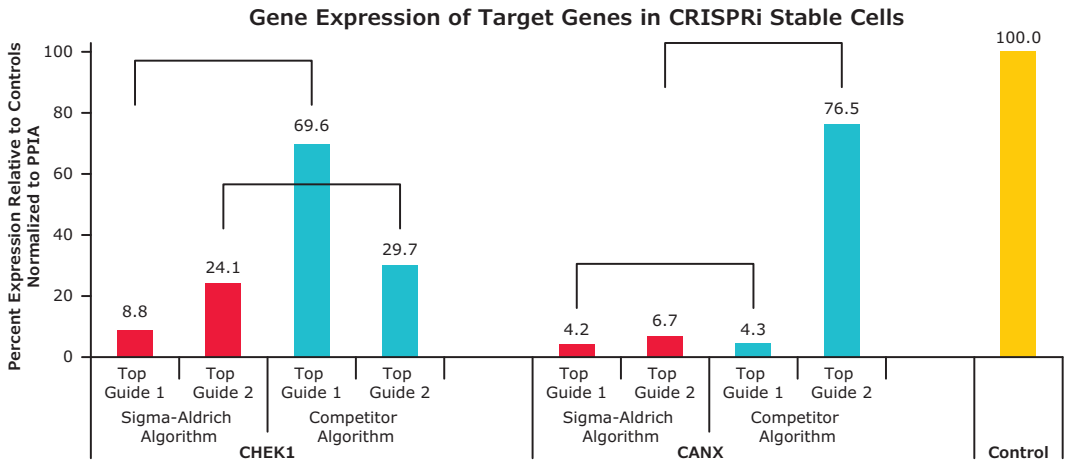
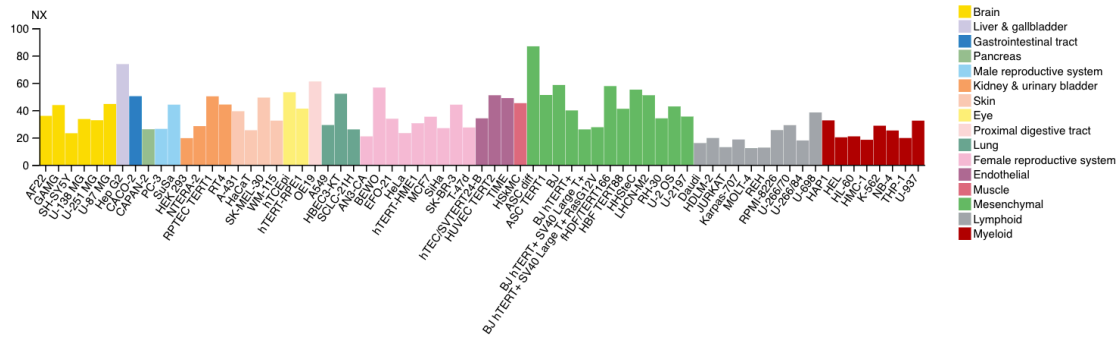


Figure 3. Comparison of expression levels using the Sigma-Aldrich® CRISPRi system compared to competitors. Graph shows relative expression levels of CHECK1 and CANX, two difficult-to-repress gene targets in stable KRAB-dCas9 cells as determined using qRT-PCR, comparing the highest ranked guides from two different algorithms. The Sigma-Aldrich® CRISPRi guides showed up to 72% more efficient gene knockdown compared to competing algorithms in all sites analyzed.

Experimental Controls

It is critical in any experiment to utilize the appropriate controls. A negative, non-targeting control (NTC) ensures a baseline and prevents false positives. Positive controls are most efficient and universal when they exhibit consistent expression across multiple cell and tissue types. The Sigma-Aldrich® RAB1A positive control provided the best generalized knockdown across multiple cell lines, as cited in literature by Gilbert et al.[3] as a top control target. While not necessary for negative controls, positive controls should always be validated in your individual experimental context.

A



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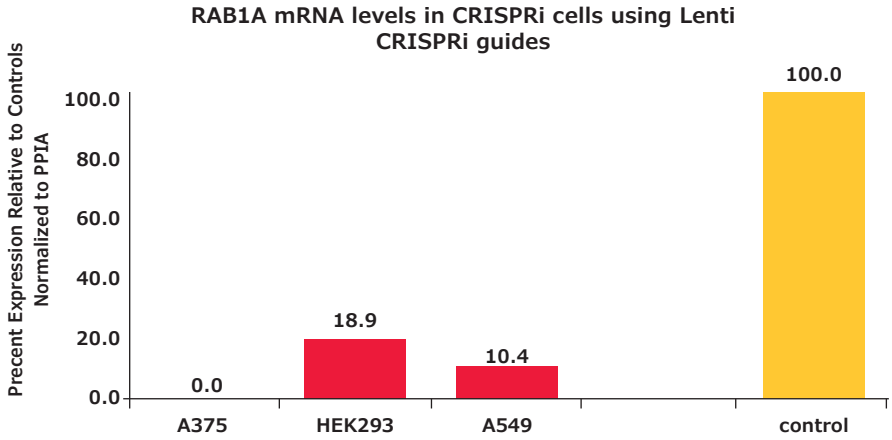

Figure 4. RNA expression and knockdown of RAB1A positive control. (A) The human protein atlas RNA expression overview. X-axis illustrates cell lines arranged by tissue type, showing consistent expression across most tissues, making it an ideal control for gene knockdown. Y-axis plots relative RNA expression levels **(B)** Sigma-Aldrich® sgRNAs were delivered via lentivirus into stable KRAB-dCas9 cell lines (A375, HEK293, A549) and assessed by qRT-PCR after 6 days. In all 3 cell lines gene knockdown was greater than 80%.

Enrichment Screening:

CRISPRi screening allows researchers to study thousands of genes at once and assess their function in a single experiment, greatly expediting the identification and validation of novel drug targets or understanding genes in their underlying role in health and disease. We performed a genome-scale enrichment screen using CRISPRi to implicate new genes and pathways responsible for resistance to Paclitaxel (PAX)-mediated cell death in human lung adenocarcinoma (A549) cells. Cells were exposed to varying doses of PAX to achieve enrichment of resistant cells. We focused our approach using SigmaAldrich® CRISPRi cancer and apoptosis subpools (CRISPRI02-1KT). A549 cells stably expressing KRAB-dCas9 were transduced with a pool of lenti guide RNAs for two replicates. The 4nM low dose allowed for enrichment by driving varying rates of proliferation. The 25nM high dose killed off most cells, and those remaining proliferated relatively normally to achieve optimal enrichment.

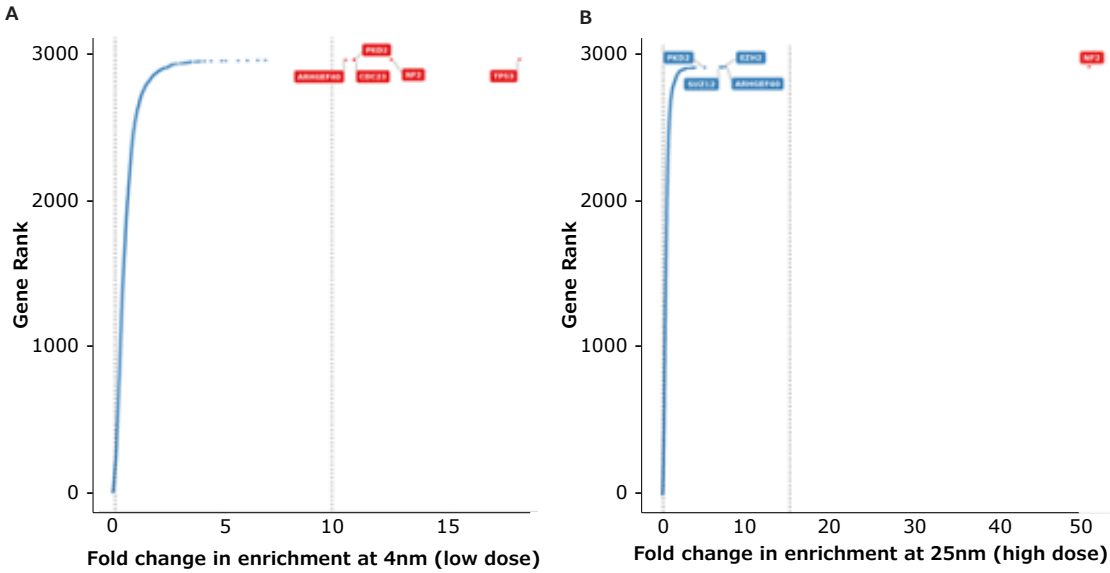

Figure 5. Genomic enrichment screen for Paclitaxel (PAX) resistance using CRISPRi. A549 NSCLC cells stably expressing KRAB-dCas9 were transduced with a CRISPRi gRNA library subpool containing up to 5 guides targeting genes relevant to cancer/apoptosis. Guide enrichment was driven by resistance to varying doses of PAX compared to an untreated control. **(A)** Fold change of guides between control and 4nM, low-dose PAX-treatment. **(B)** Fold change of guides between untreated control and 25nM, high-dose PAX-treatment. The top 5 genes are indicated. Significantly enriched genes are highlighted in red. The analysis and graphs were generated using MAGeCK 0.5.9.4 and MAGeCKFlute 1.10.0 (Wang et al.[7]) with R 4.0.2, using the default parameters except for the fold change parameter, which was not log2-normalized.

Screening with CRISPRi has the potential to reveal new genes involved in relevant drug and cellular phenotypes. Our screens identified a requirement for TP53 in paclitaxel-mediated cell death, especially at the lower dose. TP53, a known tumor suppressor gene, has previously been implicated in PAX resistance.[5,6] Strikingly, we observed significant enrichment of NF2 (neurofibromatosis type 2) in both dose level treatments. NF2, which encodes the protein merlin, plays a pivotal role in tumor suppression by restricting proliferation and promoting apoptosis, and has not been previously implicated in paclitaxel-mediated cell death. Loss of NF2 can compromise Hippo pathway activity, potentially leading to multidrug resistance.[8] The cellular context through which NF2 suppression promotes Paclitaxel resistance may prove important for overcoming resistance to anti-cancer therapies. CRISPRi, especially when used in combination with complementary screening approaches (e.g. CRISPR-KO, CRISPRa, shRNA), represents a powerful strategy to discover and dissect genes and pathways that govern cellular function.

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