CRISPR-mediated transcriptional repression with a novel dCas9 fusion protein and synthetic guide RNAs

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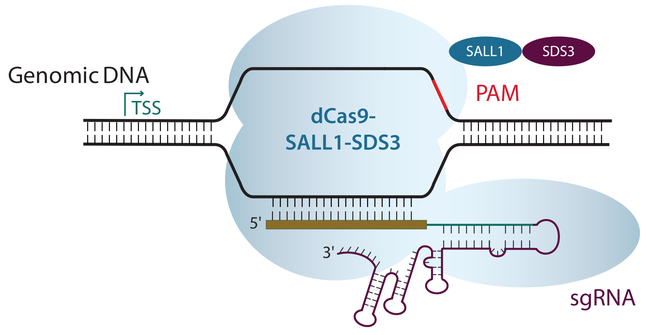
Abstract

The CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 (CRISPR-associated 9 proteins) system derived from *Streptococcus pyogenes* commonly used for genome editing has also been adapted for transcriptional modulation and epigenetic engineering, such as CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi). For CRISPRi, the guide RNA forms a complex with a nuclease-deactivated Cas9 (dCas9, D10A, and H840A), fused to one or more transcriptional effectors. The guide RNAs target the region proximal to and downstream of the transcriptional start site (TSS) to down-regulate the expression of the target gene. First-generation CRISPRi systems utilize the Krüppel associated box (KRAB) domain from zinc finger protein 10 (KOX1) fused to dCas9 (dCas9-KRAB) as a transcriptional repressor, an approach shown to be more target-specific than other existing technologies for gene repression. Given that this CRISPR-based approach can also result in less robust repression of the target gene(s), there is a need for identifying ways to improve its efficiency.

Here we describe a novel fusion protein for CRISPRi comprised of domains from two human transcriptional repressors, Sal-like protein 1 (SALL1) and Sin3 histone deacetylase corepressor complex component SDS3 (SDS3 or SUDS3), fused to the C-terminus of dCas9, and examine its efficacy across a range of applications and cell types. We demonstrate that chemically synthesized sgRNAs can be used in cells stably expressing dCas9-SALL1-SDS3 to achieve robust target gene repression that is consistently greater than the repression observed in cells stably expressing dCas9-KRAB. Furthermore, we show that synthetic sgRNAs can be used with in-vitro transcribed dCas9-SALL1-SDS3 mRNA for robust CRISPRi-mediated repression. CRISPRi using synthetic sgRNA guides enables rapid characterization of gene function complementary to other loss-of-function methodologies and allows for the performance of complex end-point assays and the examination of a wide range of phenotypes.

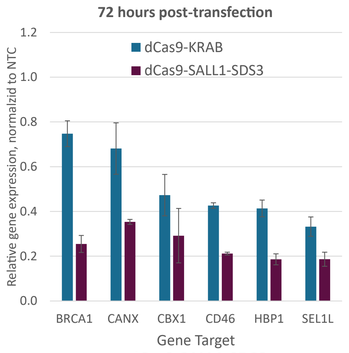
Horizon's novel CRISPRi system

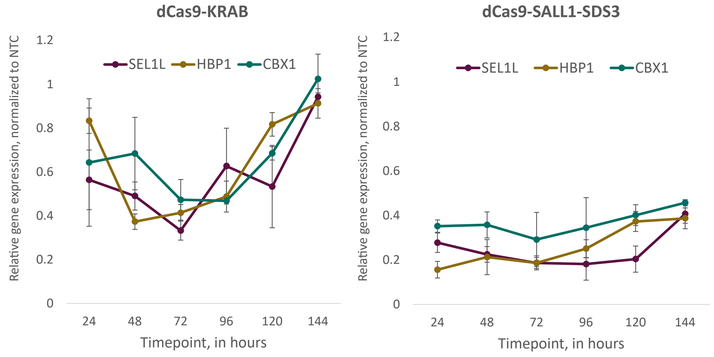


Horizon's CRISPRi system uses deactivated Cas9 (dCas9) fused to domains from the transcriptional repressors SALL1 and SDS3, dCas9-SALL1-SDS3. The single guide RNA (sgRNA) forms a complex with the fusion protein and directs it to the region immediately downstream of the target gene's transcriptional start site (TSS) to down-regulate expression.

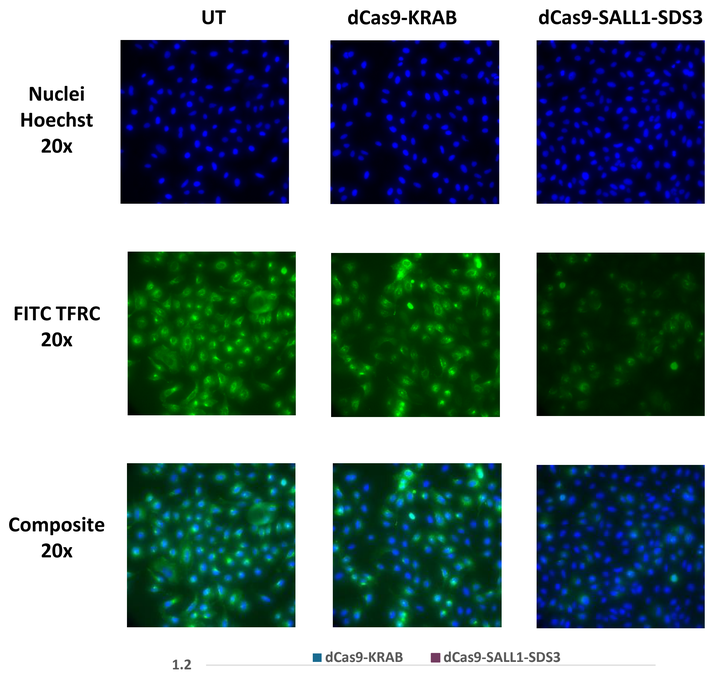
Robust transcriptional repression with dCas9-SALL1-SDS3 and synthetic sgRNA

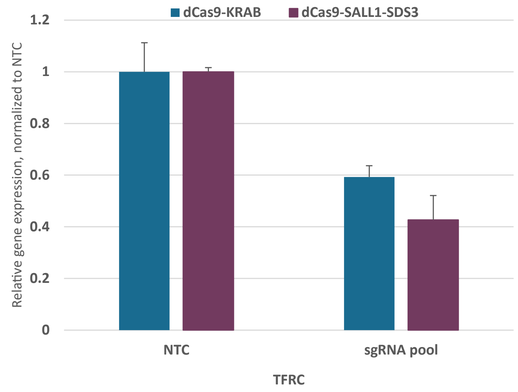
U2OS cells stably expressing integrated dCas9-KRAB or dCas9-SALL1-SDS3 were transfected with pools of synthetic sgRNA (25 nM) using DharmaFECT 4 Transfection Reagent. Cells were harvested at 24, 48, 72, 96, 120, and 144 hours post-transfection, total RNA was isolated, and relative gene expression was measured using RT-qPCR. The relative expression of each target gene was calculated with the ΔΔCq method using GAPDH as the housekeeping gene and normalized to a non-targeting control (NTC). dCas9-SALL1-SDS3 consistently enacted greater repression across all timepoints and gene targets than dCas9-KRAB.





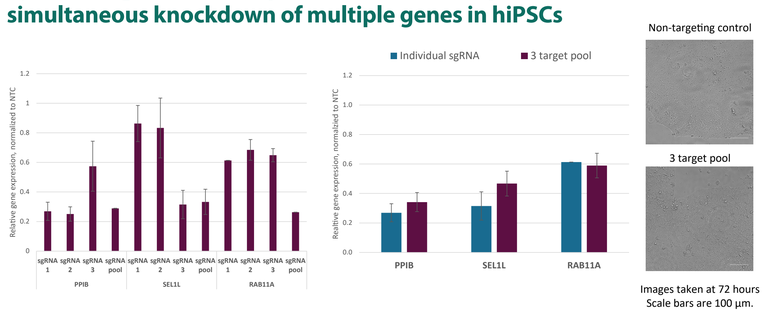
Immunofluorescence analysis indicates protein level knockdown





Functional knockdown of TFRC was assessed in U2OS cells constitutively expressing either dCas9-KRAB or dCas9-SALL1-SDS3 under the control of the hEF1α promoter. Cells were transfected with a 25 nM sgRNA pool containing three sgRNAs targeting TFRC using DharmaFECT 4 Transfection Reagent. At 72 hours post-transfection the cells were split, and at 96 hours post-transfection cells were fixed and stained with a primary antibody targeting TFRC, and an Alexa Fluor 488 conjugated fluorescent secondary antibody. Hoechst stain was used to identify nuclei. RNA was isolated from a duplicate plate and relative gene expression was measured using RT-qPCR. dCas9-SALL1-SDS3 mediates more target gene knockdown at both the mRNA and protein levels.

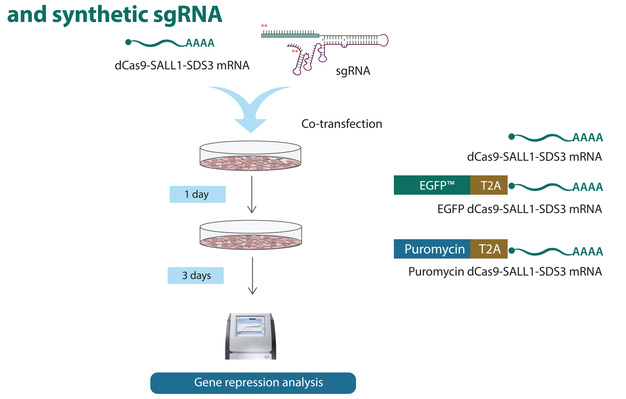
Synthetic sgRNAs can be pooled to enhance repression or enable simultaneous knockdown of multiple genes in hiPSCs

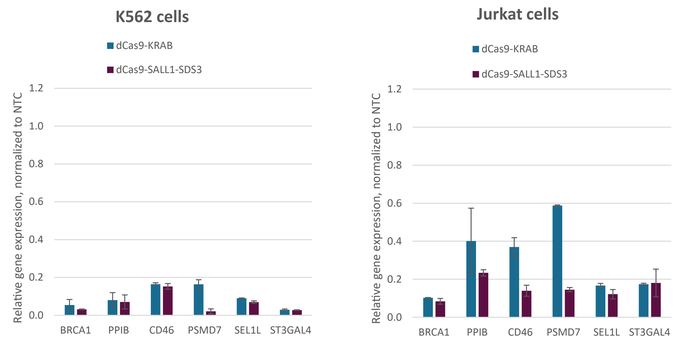


Images taken at 72 hours
Scale bars are 100 µm.

Individual CRISPRmod CRISPRi sgRNAs can be pooled together in a single reagent to achieve enhanced target gene repression or multiplexed to enable the simultaneous repression of multiple genes. WTC-11 human iPS cells stably expressing integrated dCas9-SALL1-SDS3 were nucleofected with synthetic sgRNA targeting PPIB, SEL1L, and RAB11A via a Lonza 96-well Shuttle system. The most active pre-designed sgRNA for each gene target was selected and used either individually or as part of a multi-target pool at a concentration of 3 µM per guide. Cells were harvested 72 hours post-nucleofection, total RNA was isolated, and relative gene expression was measured using RT-qPCR. The relative expression for each target gene was calculated with the ΔΔCq method using ACTB as the housekeeping gene and normalized to a non-targeting control (NTC). Three genes were simultaneously repressed without a substantial decrease in target gene repression or marked changes in cell viability and morphology.

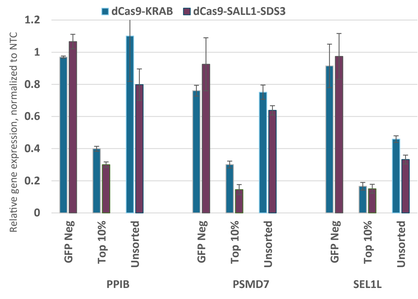
CRISPRi gene knockdown with co-delivery of dCas9-SALL1-SDS3 mRNA and synthetic sgRNA

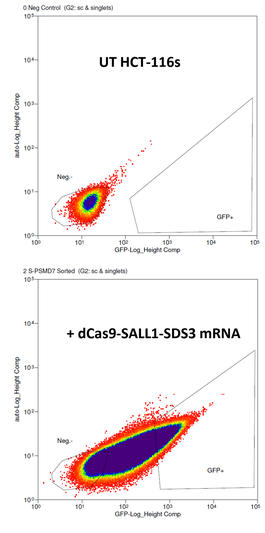




dCas9-SALL1-SDS3 mRNA enables rapid CRISPR-directed repression in complex cell models. Enrichment for gene repression can be achieved using dCas9-SALL1-SDS3 mRNA reagents that co-express either EGFP or puromycin resistance marker. K562 and Jurkat cells were nucleofected with dCas9-KRAB or dCas9-SALL1-SDS3 mRNA (2 µg), and pooled synthetic sgRNA (5 µM via a Lonza 96-well Shuttle system. K562 cells were harvested 48 hours post-nucleofection, Jurkat cells were harvested 72 hours post-nucleofection. Total RNA was isolated, and relative gene expression was measured using RT-qPCR

EGFP dCas9-SALL1-SDS3 mRNA allows for FACS enrichment in difficult-to-transfect cell models





HCT-116 cells were co-transfected with EGFP-dCas9-KRAB or EGFP dCas9-SALL1-SDS3 mRNA (2.5 µg), and pooled sgRNA targeting PPIB, PSMD7, or SEL1L (25 nM) using DharmaFECT Duo Transfection Reagent. At 24 hours post-transfection cells were trypsinized and FACS was performed to sort the cells into two categories: GFP negative (GFP Neg), and top 10% GFP expressing (Top 10%). Cells were harvested after 24 hours of recovery, total RNA was isolated, and relative gene expression was measured using RT-qPCR. Use of dCas9-SALL1-SDS3 mRNA resulted in increased target gene repression across all gene targets in both enriched and unsorted populations.

Conclusions

- Transcriptional repression achieved with dCas9-SALL1-SDS3 is consistently greater than that achieved with dCas9-KRAB.
- Potent down-regulation of target genes with CRISPRi can be achieved with chemically synthesized sgRNA enabling loss-of-function studies.
- The use of synthetic sgRNA is ideal for simultaneous repression of multiple genes or co-transfection with dCas9-SALL1-SDS3 mRNA for rapid CRISPRi studies.
- EGFP dCas9-SALL1-SDS3 mRNA enables researchers to enrich for knockdown in difficult-to-transfect cell models.

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