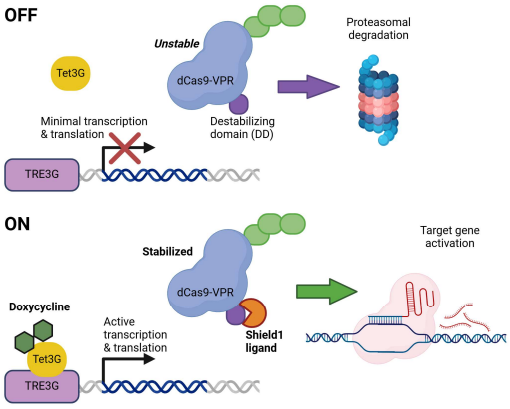
Tight temporal control over CRISPR activation through dual transcriptional and post-translational regulation of dCas9-VPR expression

Clarence Mills, Brian Ziemba, Johanna De Castro Arce, Allyson Malloy, Andrew Riching, Emily Anderson, Josien Levenga

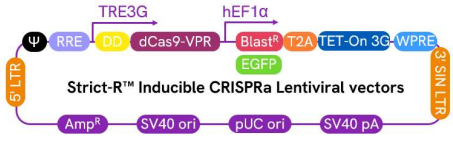
1 Abstract

CRISPR-Cas9 has been widely adapted for use in transcriptional modulation and epigenetic engineering with deactivated Cas9 (dCas9) systems to enable CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) applications. However, few of these systems provide researchers with the ability to control the timing of CRISPR-mediated transcriptional modulation. Here we describe a novel small molecule-inducible system for potent, stringently regulated CRISPR activation. The dCas9-VPR CRISPRa effector is fused to an FKBP12-derived destabilizing domain and expressed from a Tet-inducible promoter (TRE3G) to prevent transcription and stable translation of dCas9-VPR in the absence of the doxycycline and Shield1 ligand. We show that in combination, the Tet-On system and the FKBP12-derived destabilizing domain minimize basal target gene activation (leakiness) while maintaining robust induction across gene targets and cell types. Furthermore, the inclusion of the destabilizing domain enables a degree of temporal control that is not possible with the Tet-On system alone. We then demonstrate how this dual regulated dCas9-VPR can be applied in human induced pluripotent stem cells (hiPSCs) to overexpress key proneural factors. In addition, we present a proof-of-concept model where siRNA and CRISPRa are simultaneously used to suppress or enhance target genes governing drug resistance in populations of drug-resistant cancer cell lines. By strategically combining a selection of these siRNA and CRISPRa resistance targets, we observe a synergistic effect, further restoring drug sensitivity in these resistant cell lines and underscoring how siRNA and CRISPRa can be paired within a single experiment for pathway-based screening and drug target identification.

2 Dharmacon™ Strict-R™ Inducible CRISPRa System



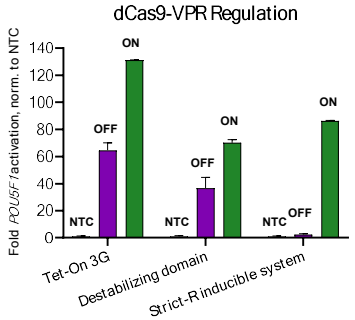
In the absence of Doxycycline and Shield1, the system is "**OFF**". Leaky bursts of transcription from the TRE3G promoter result in the translation of dCas9-VPR fused to a FKBP12-derived destabilizing domain that tags the protein for rapid proteasomal degradation minimizing background activation (leakiness).
ON: The addition of doxycycline induces potent transcription from the TRE3G promoter and the addition of Shield1 stabilizes dCas9-VPR thereby enabling robust target gene activation in the presence of a gene-specific sgRNA. *Diagram created with BioRender.com.*



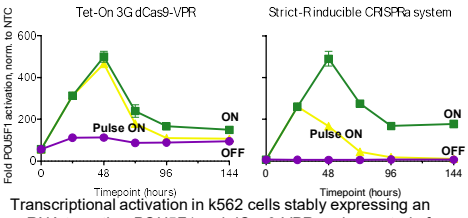
Schematic map of the Dharmacon Strict-R Inducible CRISPRa Lentiviral vectors and CRISPRmod CRISPRa Lentiviral sgRNA vector.

CRISPRa Lentiviral sgRNA

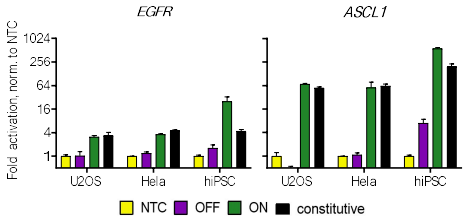
3 Tighter temporal control of CRISPR activation



Transcriptional activation in k562 cells stably expressing an sgRNA targeting *POU5F1* and dCas9-VPR under control of the respective system. "ON" cell populations (**green**) were induced for 24 hours prior to RT-qPCR analysis.

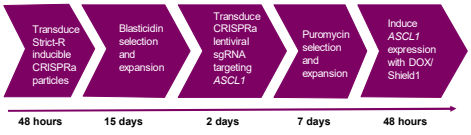


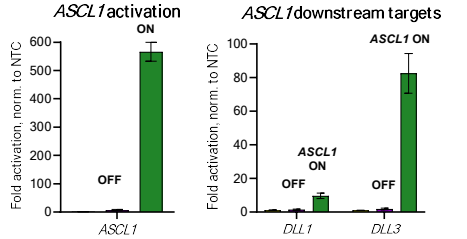
Transcriptional activation in k562 cells stably expressing an sgRNA targeting *POU5F1* and dCas9-VPR under control of the respective system. Induction was maintained throughout the study by replenishing small-molecule containing media every 48 hours (ON, **green square**). Matched cell populations (Pulse ON, **yellow triangle**) were induced for 24 hours and then washed and subsequently cultured in media without doxycycline or Shield1.



Transcriptional activation of *EGFR* and *ASCL1* in U2OS, Hela, and human induced pluripotent stem cells (hiPSC) expressing either the Strict-R Inducible dCas9-VPR system or constitutive dCas9-VPR system (**black**). "ON" cells (**green**) were induced with 250 nM Shield1 and 500 ng/ mL doxycycline for 48 hours prior to RT-qPCR analysis.

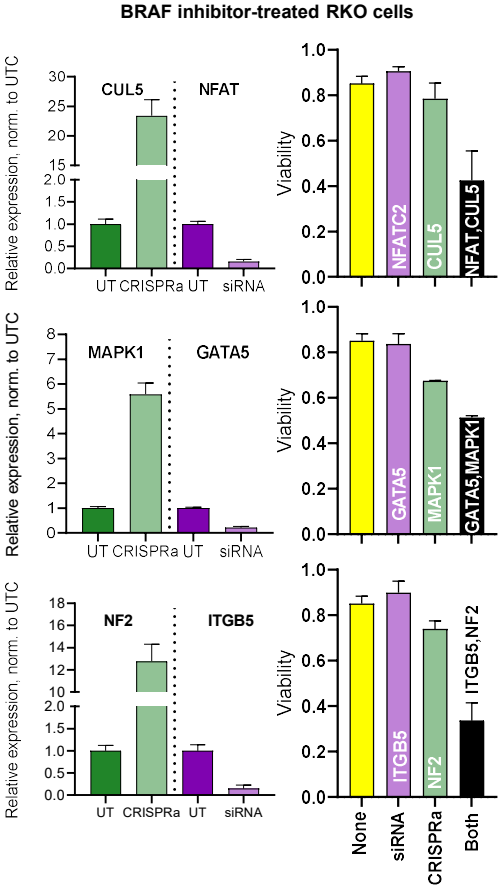
4 Small molecule activation of proneural factors in hiPSCs





Transcriptional activation in hiPSCs expressing the Strict-R inducible CRISPRa system and an sgRNA targeting *ASCL1*. "ON" cells (**green**) were induced for 2 days prior to gene expression analysis of *ASCL1* and its downstream Delta gene targets *DLL1* and *DLL3*. Activation of Delta genes by proneural transcription factors such as ASCL1 is an evolutionarily conserved step in neurogenesis that results in activation of Notch signaling and maintenance of an undifferentiated state in a subset of neural progenitors.

5 Bidirectional gene modulation via CRISPRa and siRNA co-delivery



Relative gene expression and viability in RKO (Q56P) cells stably expressing dCas9-VPR that were co-transfected with CRISPRa synthetic sgRNAs and ON-TARGETplus™ SMARTpool™ reagents targeting the listed genes. At 4 hours post-transfection, cells were treated with a BRAF inhibitor and cultured for an additional 72 hours prior to resazurin and RT-qPCR analysis.

6 Summary

- The combination of the Tet-On system and the FKBP12-derived destabilizing domain greatly reduces leakiness while enabling potent CRISPR activation with the addition of two highly cell-permeable small molecules.
- Dual transcriptional and post-translational regulation of dCas9-VPR expression provides finer temporal control over CRISPR activation.
- Small molecule induction *ASCL1* resulted in marked upregulation of downstream Delta gene targets *DLL3* and *DLL1* while low level background activation of *ASCL1* did not significantly impact *DLL1* or *DLL3* expression.
- CRISPRa and RNAi reagents can be co-delivered to mediate robust, simultaneous up-and down-regulation of multiple targets. This multimodal regulation of BRAF inhibitor resistance targets can restore drug sensitivity in a resistant human colon carcinoma cell line.

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