Timed induction of dCas9-VPR expression for tight and temporal control of transcriptional activation

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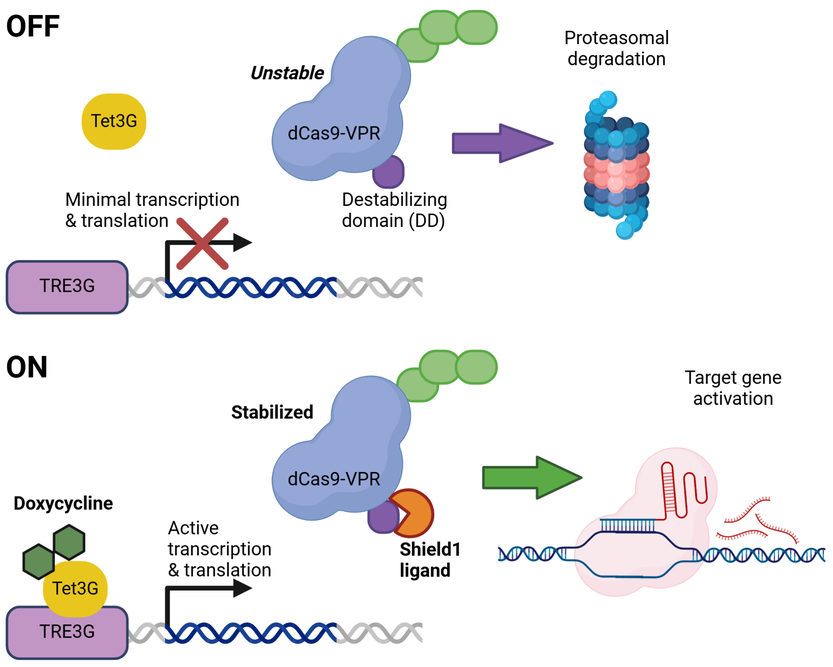
1 Abstract

CRISPR-Cas9 has become a powerful tool for genetic editing across various organisms and application spaces. Shortly after its discovery, the CRISPR system was innovated into new tools that go beyond genomic double stranded breaks: CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa). These tools allow researchers to discern the molecular mechanisms of gene repression or activation. However, few of these systems provide researchers the ability to control the timing of CRISPR-mediated transcriptional modulation. To address the need for temporal control of genetic modulation, we developed a new small molecule-inducible system for potent, stringently regulated CRISPR activation: Dharmacon™ Strict-R™ Inducible CRISPRa Lentiviral System. Here, the dCas9-VPR CRISPRa effector is fused to an FKBP12-derived destabilizing domain (degron) and expressed from a Tet-inducible promoter to prevent transcription and stable translation of dCas9-VPR in the absence of the doxycycline and Shield1 ligand. We show that this combination minimizes basal target gene activation (leakiness) while maintaining robust induction across gene targets and cell types. Furthermore, the destabilizing domain enables temporal control that was not possible with the Tet-On system alone. Finally, we demonstrate how this dual regulated dCas9-VPR can be applied in human induced pluripotent stem cells (hiPSCs) by overexpressing key proneural factors. This system provides researchers with validated reagents for temporal control across various CRISPR systems, enabling precise investigation of time-dependent gene modulation mechanisms that will accelerate discoveries in developmental biology, disease modelling, and therapeutic applications.

2 Dharmacon™ Strict-R™ Inducible CRISPRa System



OFF

- **Absence** of doxycycline and Shield1

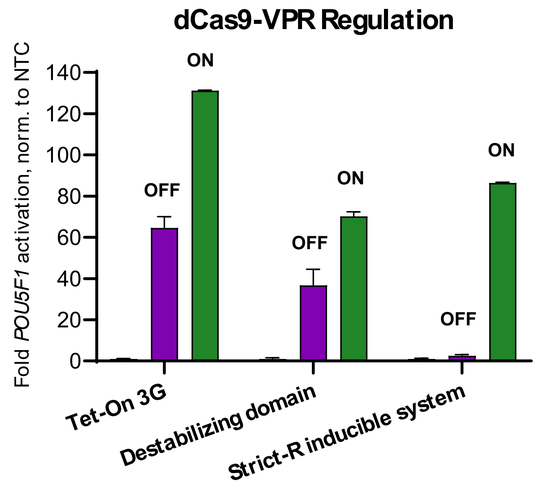
→Leaky bursts of transcription from the TRE3G promoter result in the translation of dCas9-VPR fused to a FKBP12-derived destabilizing domain that tags the protein for rapid proteasomal degradation, minimizing background activation (leakiness).

ON

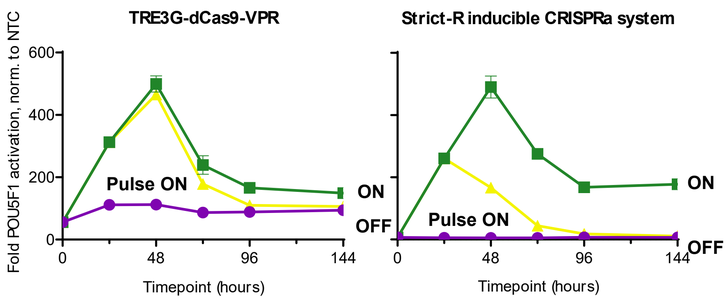
- **Presence** of doxycycline and Shield1

→ 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.*

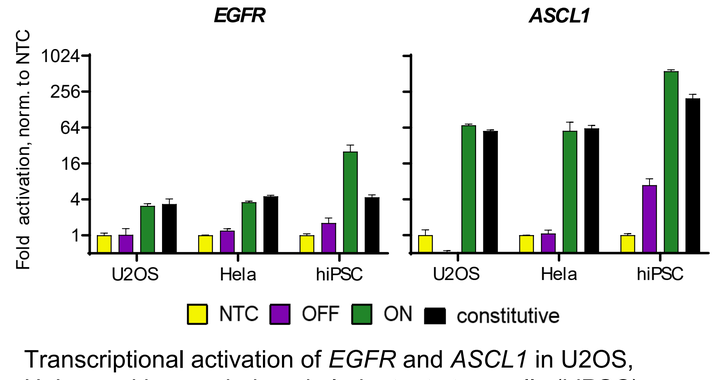
3 Tighter temporal control over CRISPR activation



Transcriptional activation in **K562 cells** stably expressing an sgRNA targeting ***POU5F1*** and dCas9-VPR under control of each respective system. "ON" cell populations (**green**) were induced for 24 hours prior to RT-qPCR analysis.

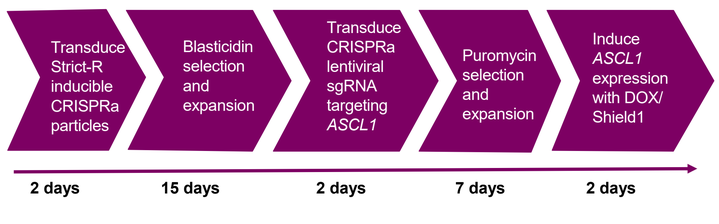


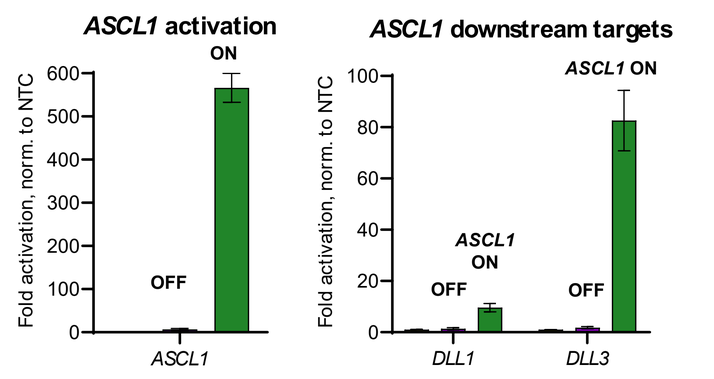
Transcriptional activation in **K562 cells** stably expressing an sgRNA targeting ***POU5F1*** and dCas9-VPR under control of each respective system. Induction was maintained throughout the study by replenishing small-molecule containing media every 48 hours (ON, **green square**). Matched cell populations (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.

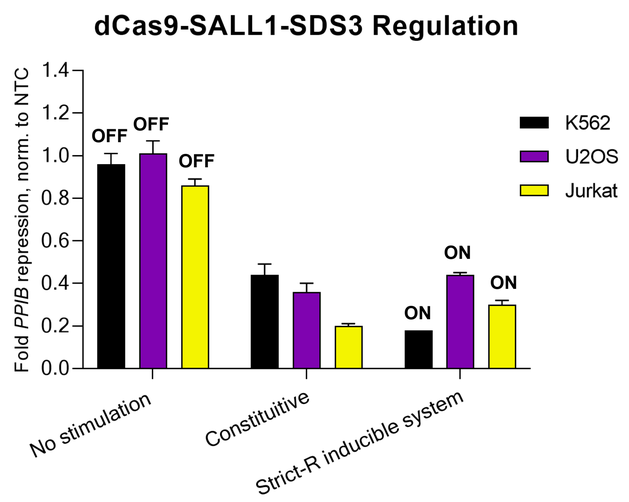
4 Small molecule activation of proneural factors in hiPSCs





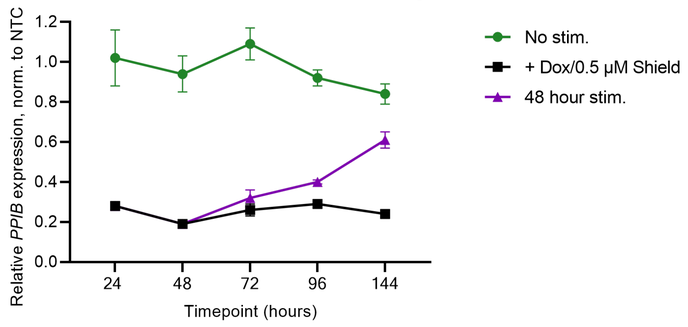
Transcriptional activation in hiPSCs expressing the Strict-R inducible CRISPRa system and an sgRNA targeting *ASCL1.* "ON" cells (**green**) were induced for 48 hours prior to gene expression analysis of *ASCL1* and its downstream Delta gene targets *DLL1* and *DLL3*. Activation of Delta genes by proneural 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 Tighter temporal control over CRISPR interference



Transcriptional repression across three cell lines stably expressing an sgRNA targeting ***PPIB*** and dCas9-SALL1-SDS3 CRISPRi platform under control of the constitutive or Strict-R inducible system. "ON" cell populations were induced for 48 hours prior to RT-qPCR analysis. Knockdown comparison to a "constitutive" promoter, which is always in the "ON" state shows comparable knockdown efficiency.

Timecourse of dCas9-SALL1-SDS3 regulation



Transcriptional repression of ***PPIB*** over time in **K562** cells using the inducible dCas9-SALL1-SDS3 system. Shield1 and doxycycline can be replenished continuously to maintain knockdown (**black**) or can be removed after desired knockdown period (**purple**) for restoration of *PPIB* protein. No stimulation (**green**) shows consistent expression of *PPIB* transcripts and protein.

Schematic map of the Dharmacon Strict-R Inducible CRISPRa and CRISPRi Lentiviral vectors.

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: doxycycline and Shield1.
- Dual transcriptional and post-translational regulation of dCas9-VPR expression provides temporal control over CRISPR activation.
- Small molecule induction of *ASCL1* resulted in significant upregulation of downstream Delta gene targets *DLL3* and *DLL1* while low level background activation of *ASCL1* did not significantly impact *DLL1* or *DLL3* expression.
- Small molecule induction of inducible dCas9-SALL1-SDS3 resulted in *PPIB* downregulation across three cell lines.
- The Strict-R system for CRISPR activation can be effectively applied to CRISPR interference, demonstrating the versatility and robustness of this method across different CRISPR technologies.