Christian Doppler Laboratory for Cardiac and Thoracic Diagnosis & Regeneration

MEDIZINISCHE UNIVERSITÄT WIEN

Cell

Resource

# Genome-Scale CRISPR-Mediated Control of Gene Repression and Activation

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Cell *159*, 647–661, October 23, 2014 

# Gene silencing Methodes

- siRNA

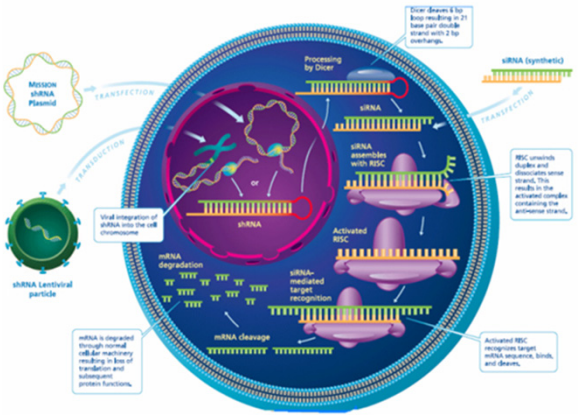
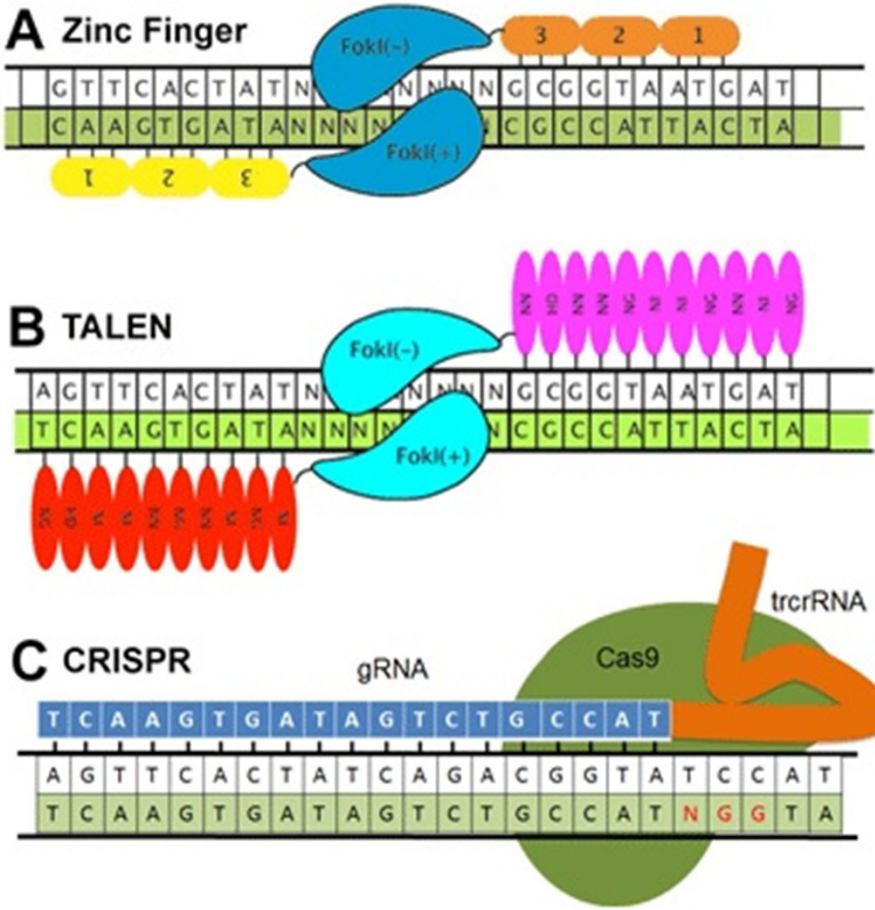
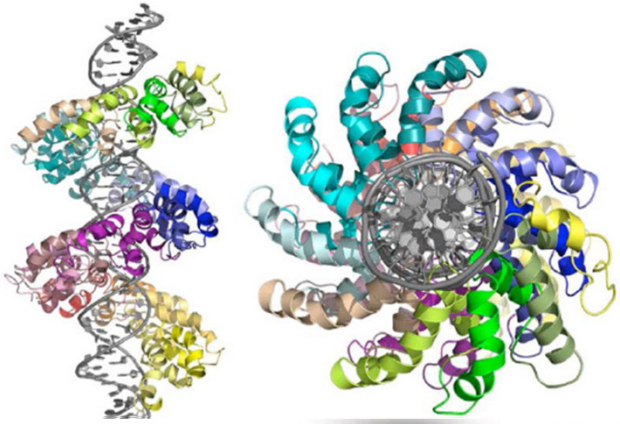
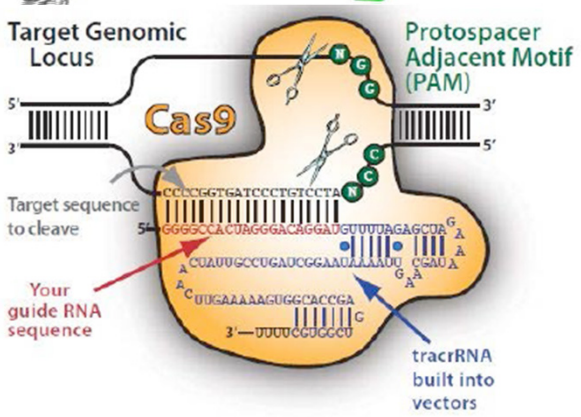



Fig: RPRG




TALE binding to DNA is very modular



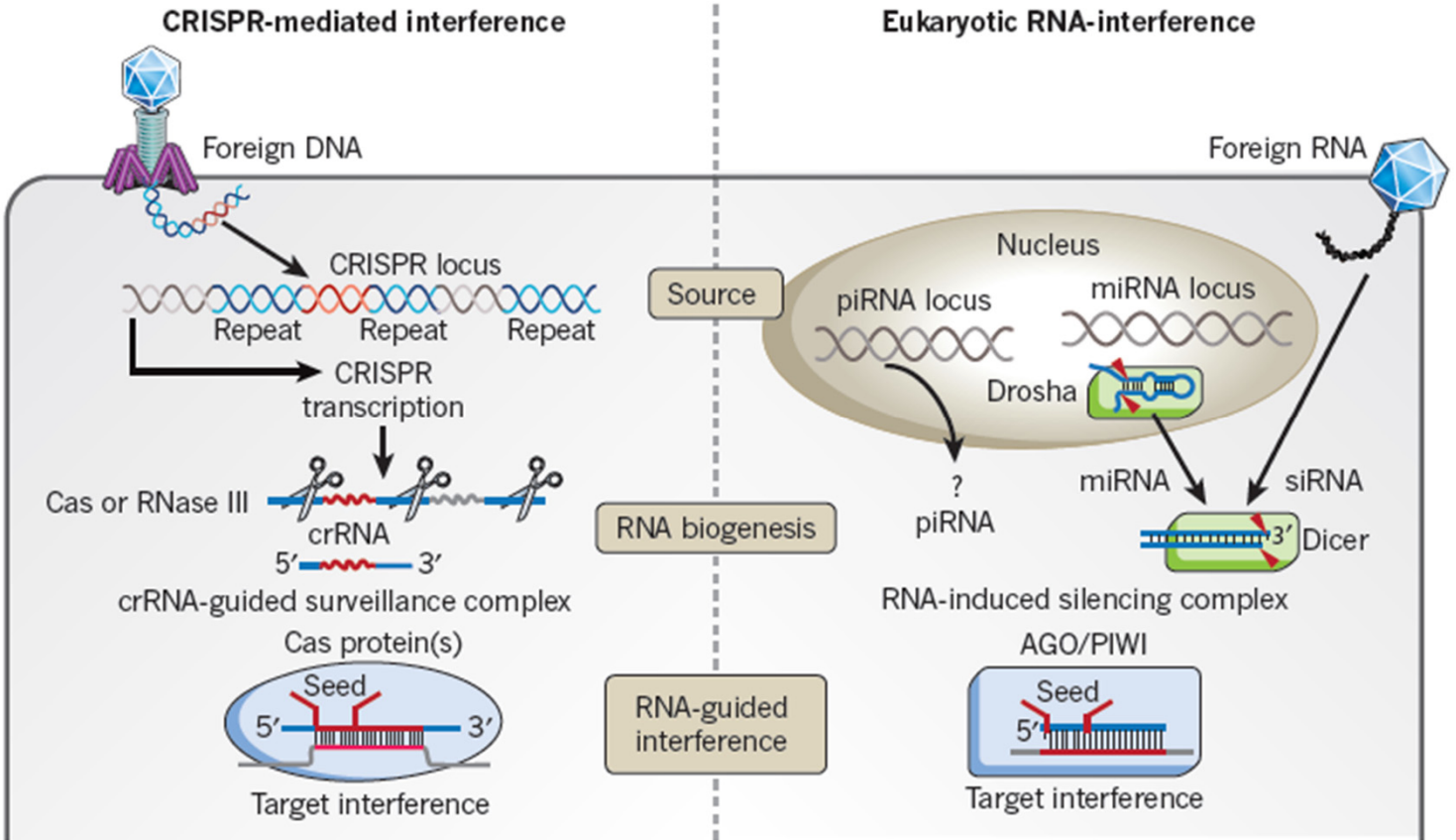



CRISPR: Courtesy:http://www.addgene.org/static/cms/images/crisp_newsletter_fig1_1.png Picture.

# CRISPR /CAS system

**CRISPR** = **C**lustered **R**egular **I**nterspaced **P**alindromic **R**epeats
**CAS**= **C**RISPR **a**ssociated **n**uclease

- Originates from a bacterial defense system
- RNA based targeting of DNA
- Ribonucleoprotein complex formed from Cas9, a crRNA and a transacting crRNA (tracrRNA) leads to site-specific DNA cleavage

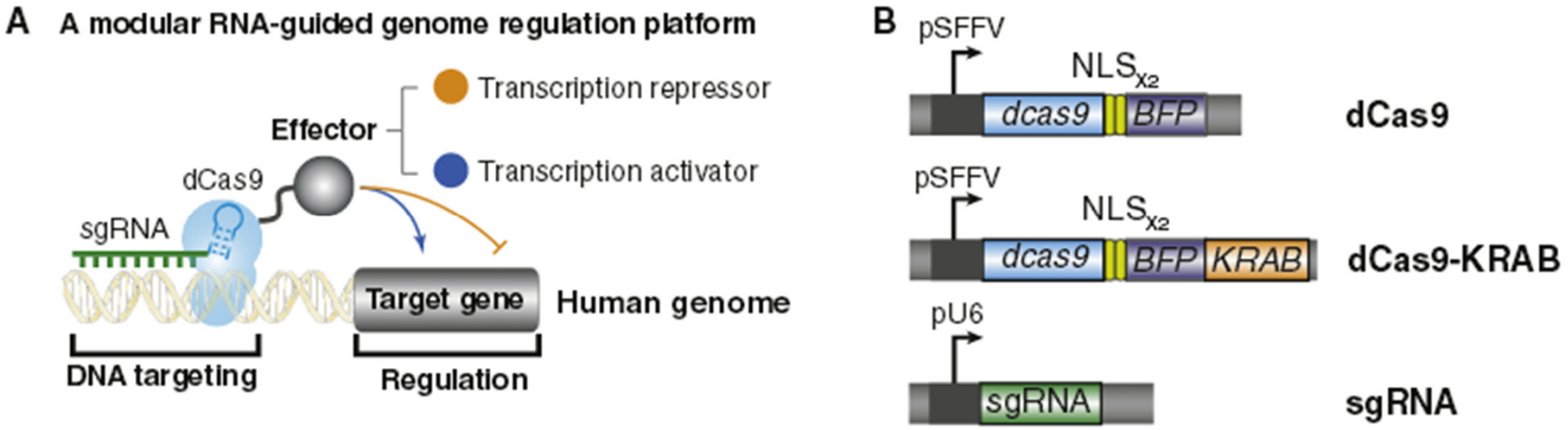


# Recent developments in the CRISPR/CAS system

**Jinek et al., 2012**: development of a chimeric single-guide RNA = **sgRNA** and a Cas9 protein – sufficient for targeted DNA binding and cleavage with the cleavage site dictated solely by complementarity of the sgRNA

**Qi et al., 2013**: endonuclease domains of Cas9 proteins can be mutated to create a programmable RNA-dependent DNA-binding protein =**dCas9**
**->** sterically block RNA-Pol. Binding or elongation

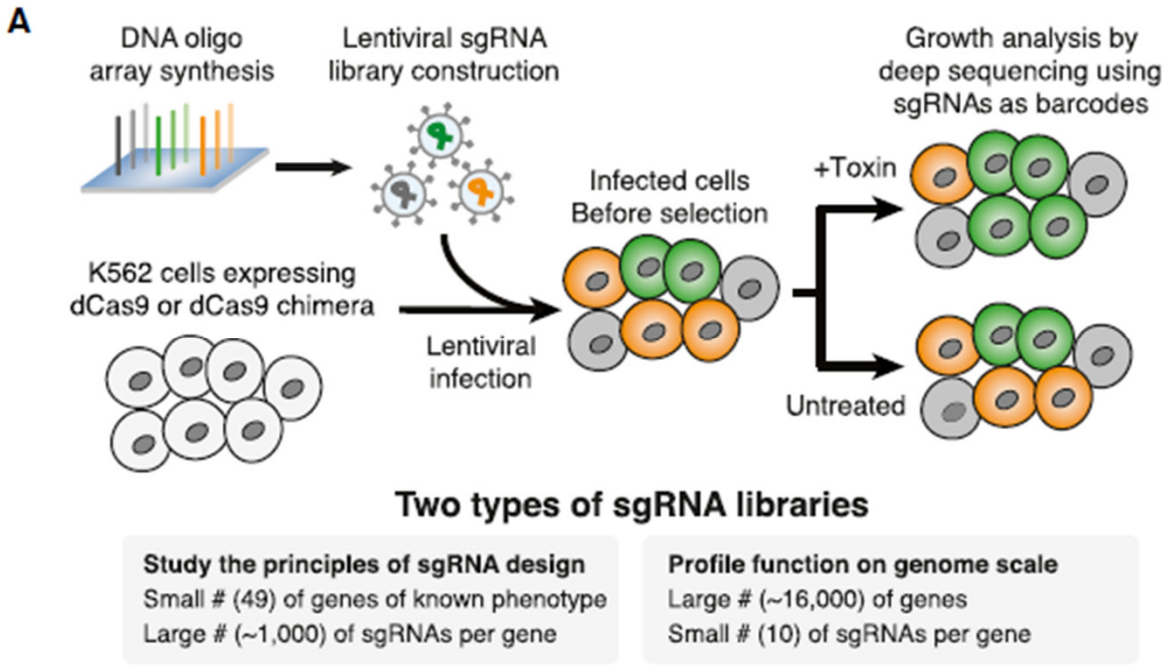
**Gilbert et al., 2013: CRISPR-Mediated Modular RNA-Guided Regulation of Transcription in Eukaryotes**
dCas9 can be used as a modular RNA-guided platform to recruit different protein effectors to DNA -> repress **(CRISPRi)** or activate **(CRISPRa)** transcription of target genes



# Aim

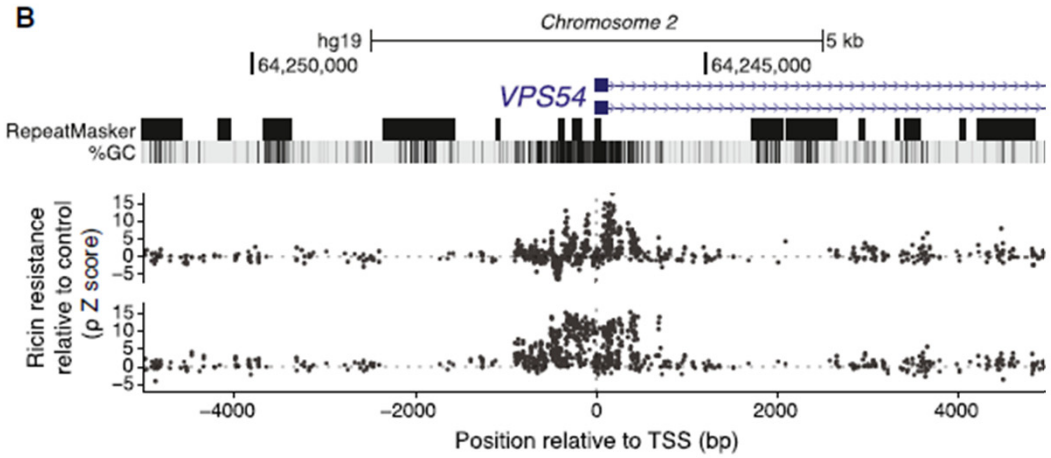
- To extract distinct rules for regions where either CRISPRi or CRISPRa maximally change the expression of endogenous genes as well as rules for predicted off-target effects

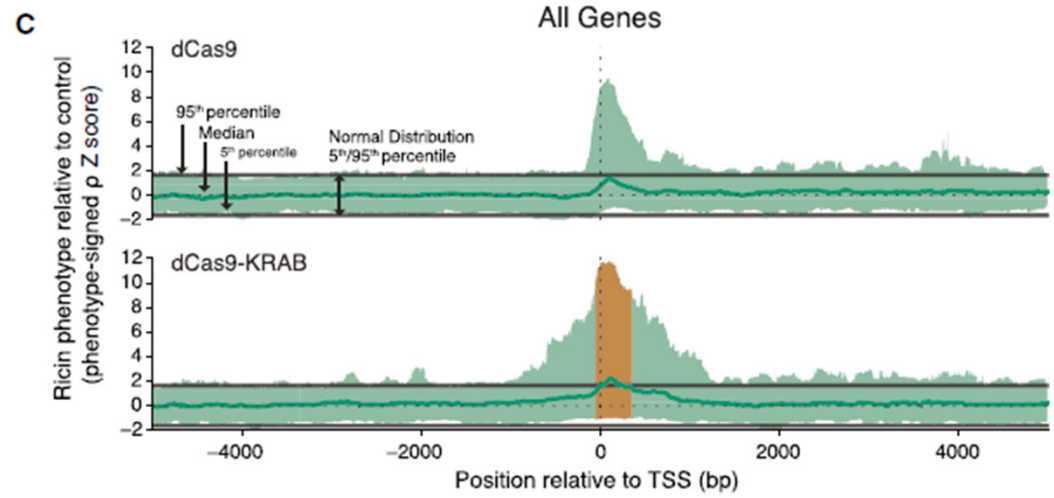
- To provide an algorithm to disign two genome-scale labraries targeting each gene with 10 sgRNAs

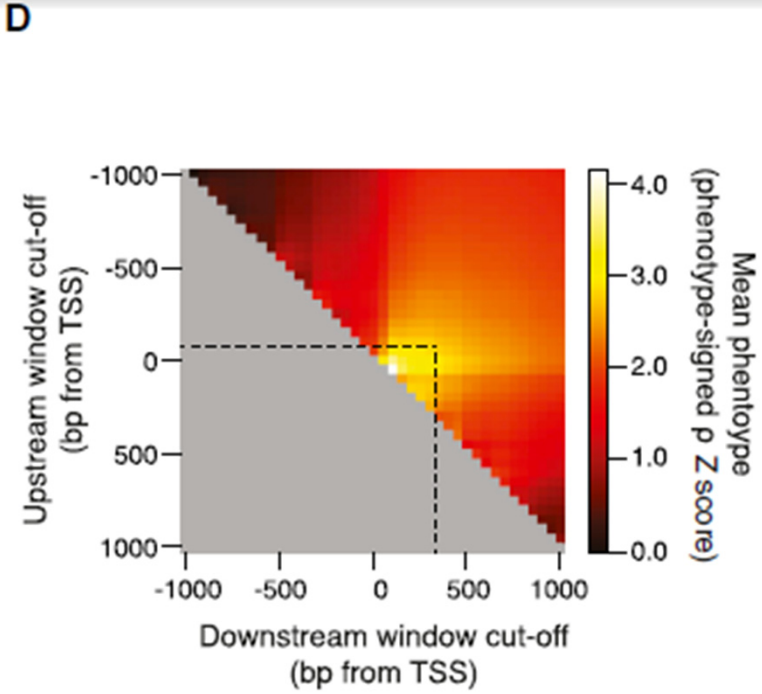



- 49 Genes that modulate cellular susceptibility to the AB toxin ricin
- Repression has a monotronic relationship with the ricin-resistance phenotype
- Ricin-resistance score to indirectly measure transcriptional repression

- determine how sgRNAs modulate cell growth (**γ**) and cellular susceptibility to ricin phenotypes (**ρ**)
- Normalized phenotype **Z score**: dividing mean phenotypes for each gene by the standard deviation of sgRNA phenotypes from the nontargeting control set.

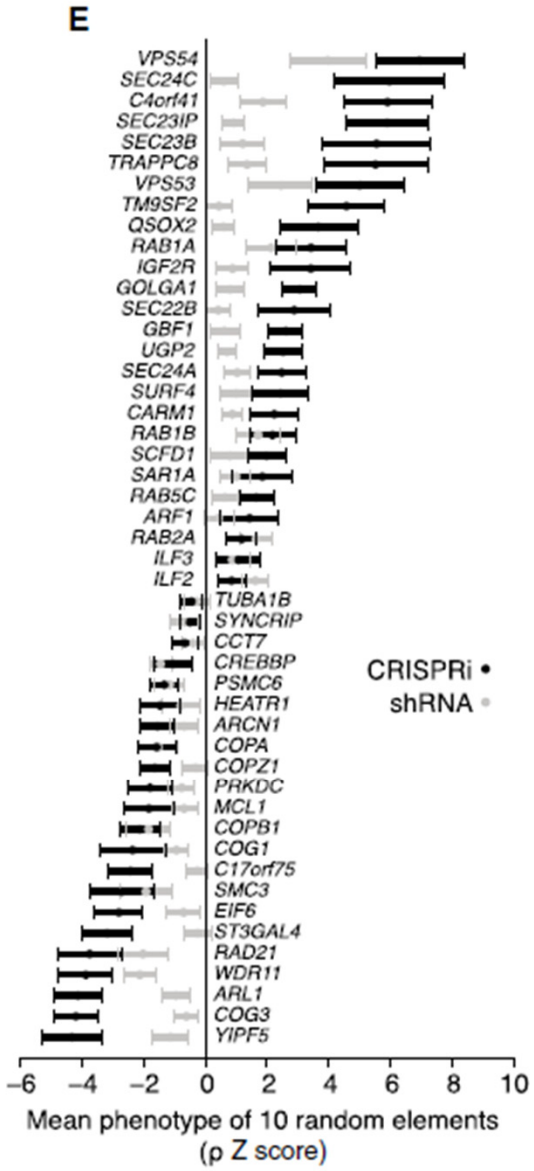


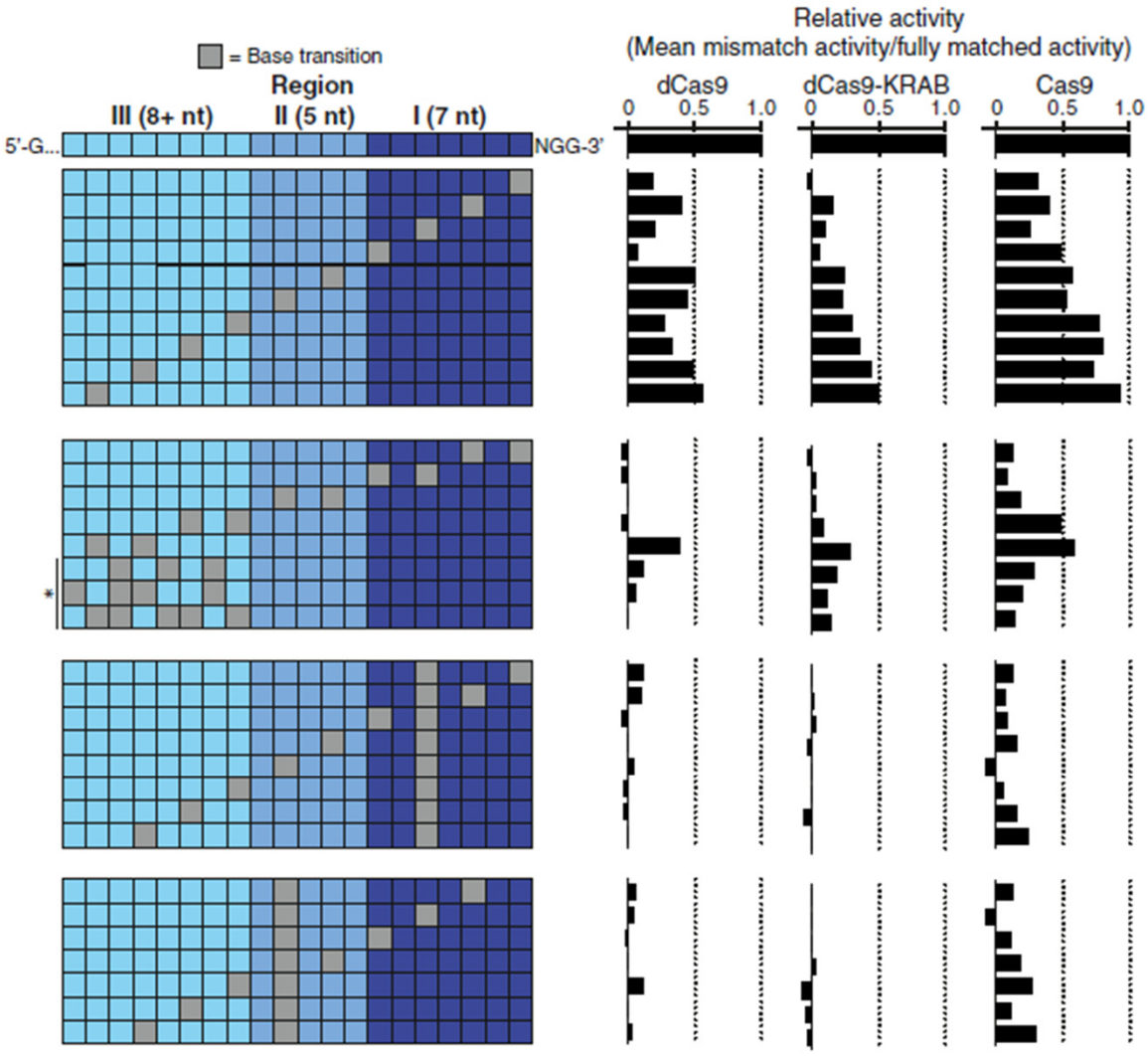




Optimal activity is achieved by the combined activity of dCas9 interference along with repression from the KRAB domain

- SgRNAs with protospacer length of 18-21 bp were sig. more active
- Nucleotide homopolymeres had a strongly neg. effect on sgRNA activity
- Neither the targeted DNA strand (+/ -) nor the GC content correlated with sgRNA activity

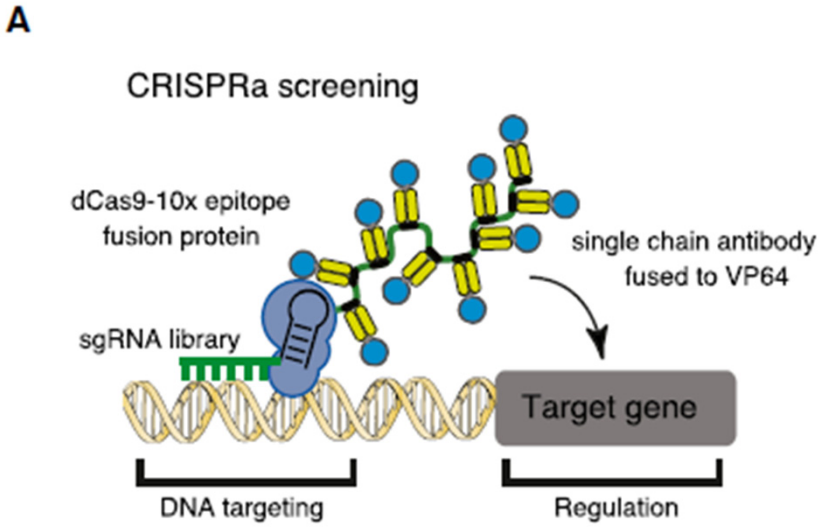


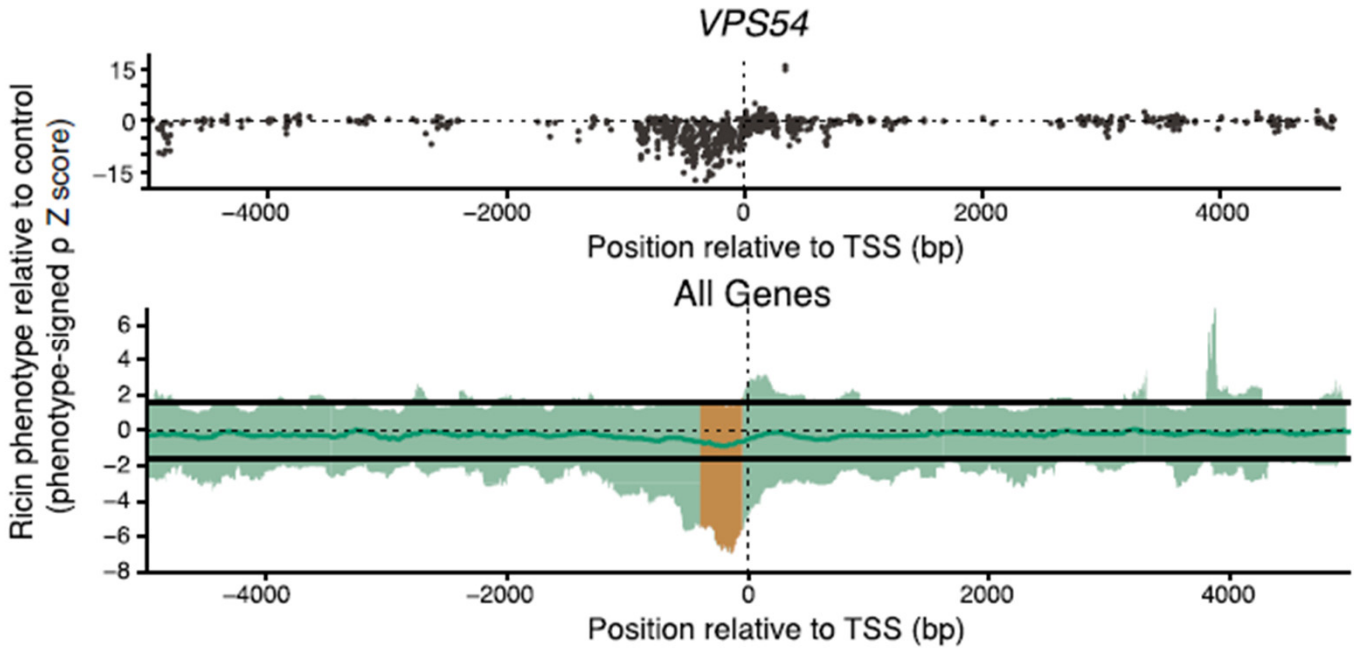

CRISPRi Z-score is stronger than seen in the shRNA library

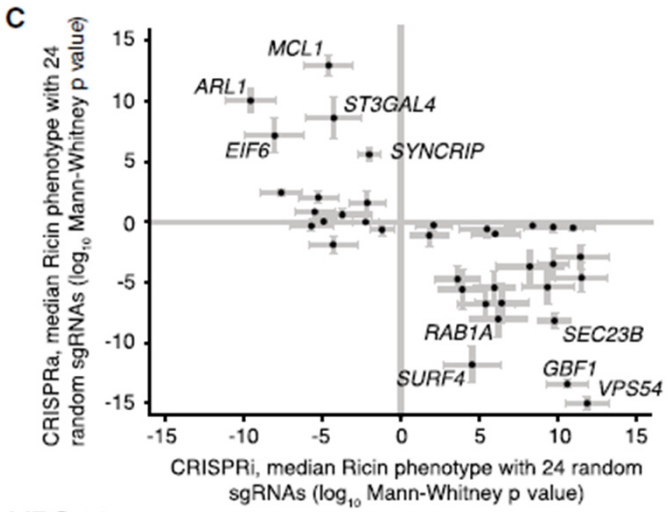
CRISPRi activity is highly sensitive to mismatches between the target DNA site and the sgRNA

# sunCas9

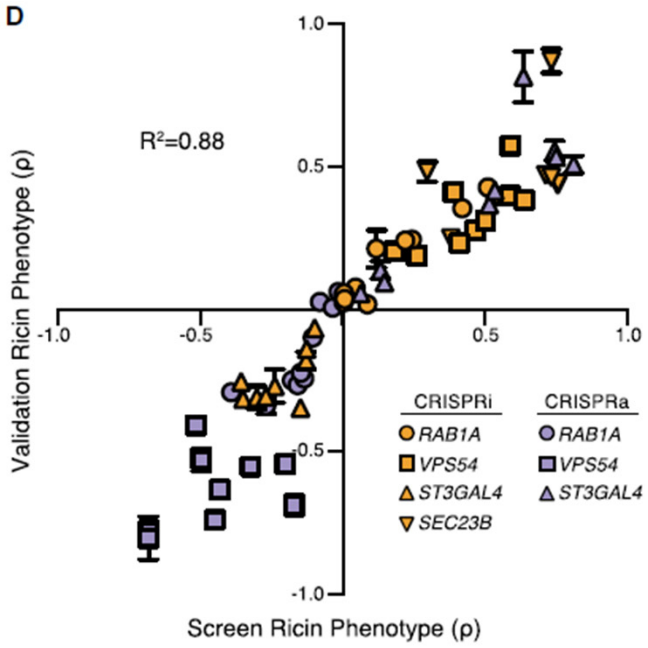


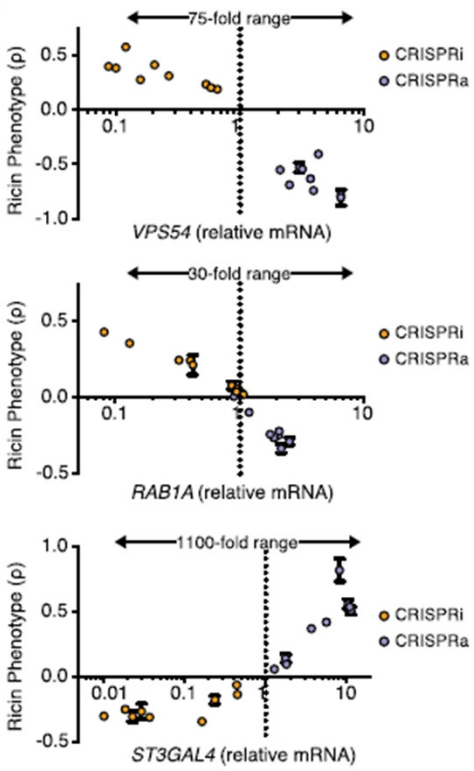

CRISPRi/a tilling screens provide rules how CRISPRi/a controls expression of endogenous genes






Anticorrelation between CRISPRa phenotypes and CRISPRi phenotypes for individual genes



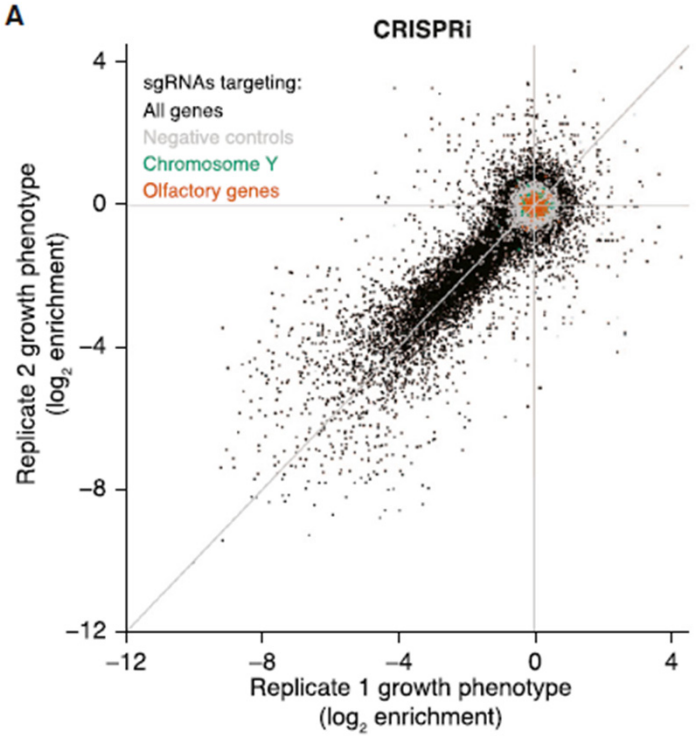

CRISPRi/a screens

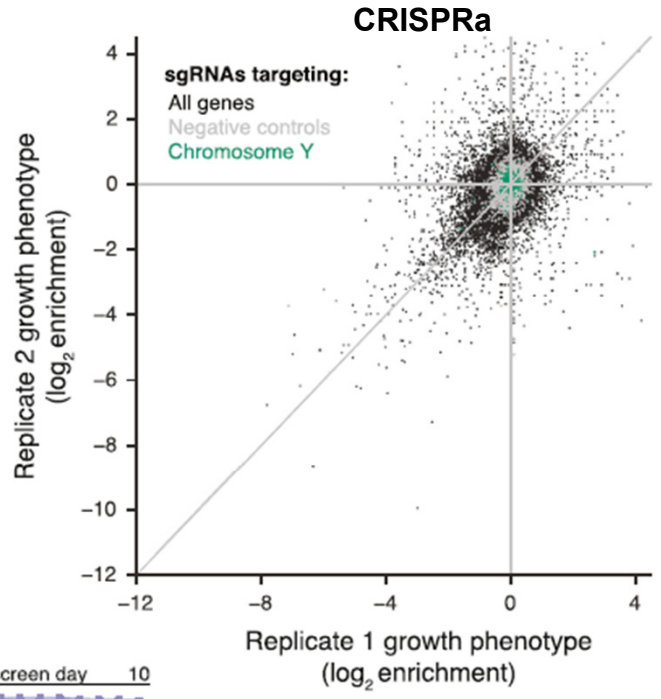
- produce reliable phenotype scores
- can activate/repress transcription over a wide dynamic range
- enabling systematic interrogation of how gene dosage controles cellular functions of interest

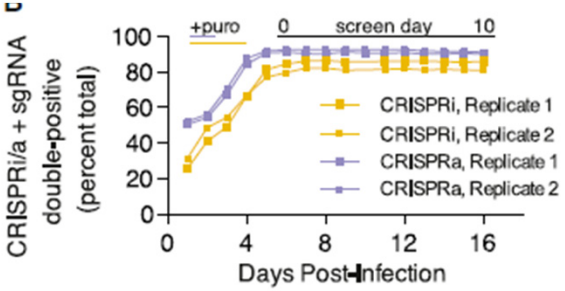
**CRISPRi sgRNA library**: targeting 15,977 human protein-coding genes (10/TSS) with 11,219 nontargeting control sgRNAs. **Total 206,421 sgRNAs**

**CRISPRa sgRNA library**: targeting 15,977 human protein-coding genes (10/TSS) with 5,968 nontargeting control sgRNAs. **Total 198,810 sgRNAs**

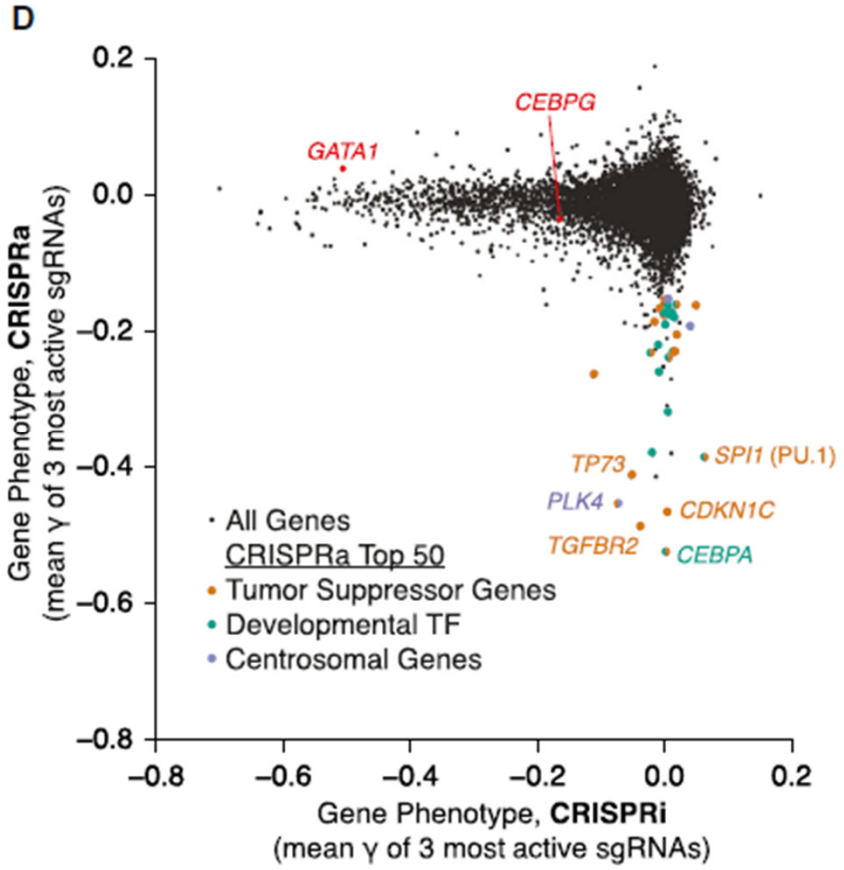
Genes essential for cell growth -> **K562** cells expressing sCas9-KRAB or sunCas9 were transduced

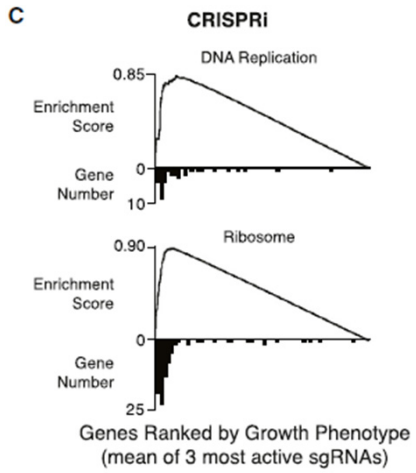


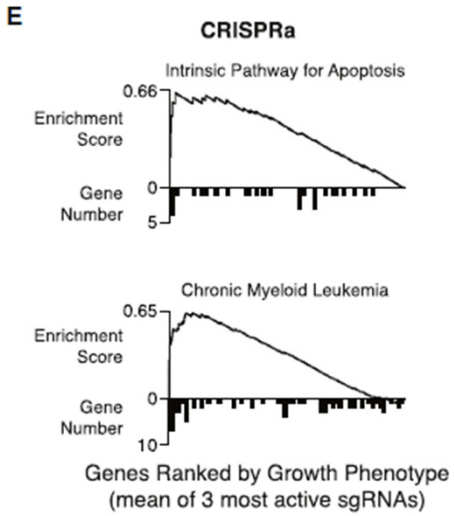



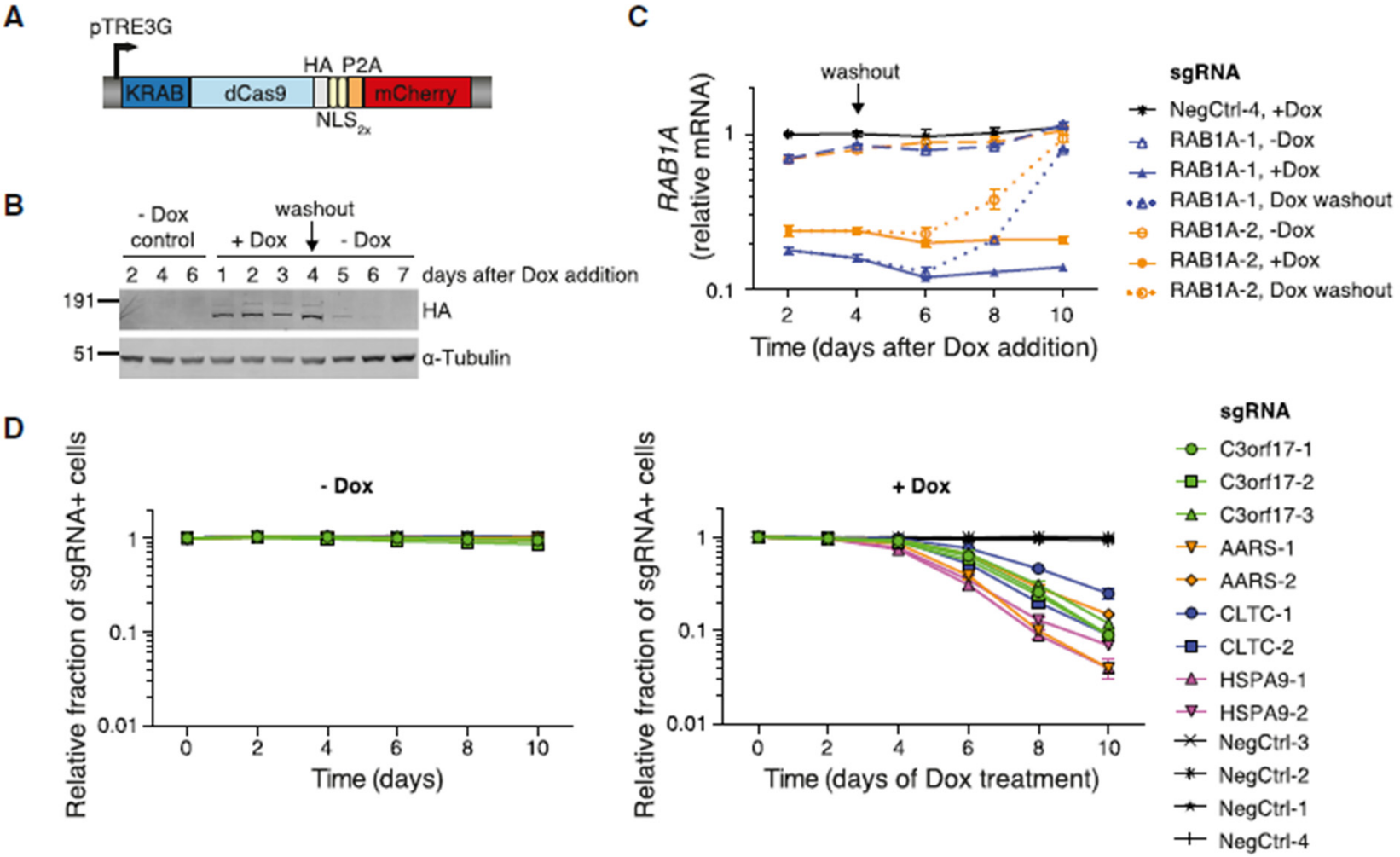

**CRISPRi/a is highly specific and nontoxic**



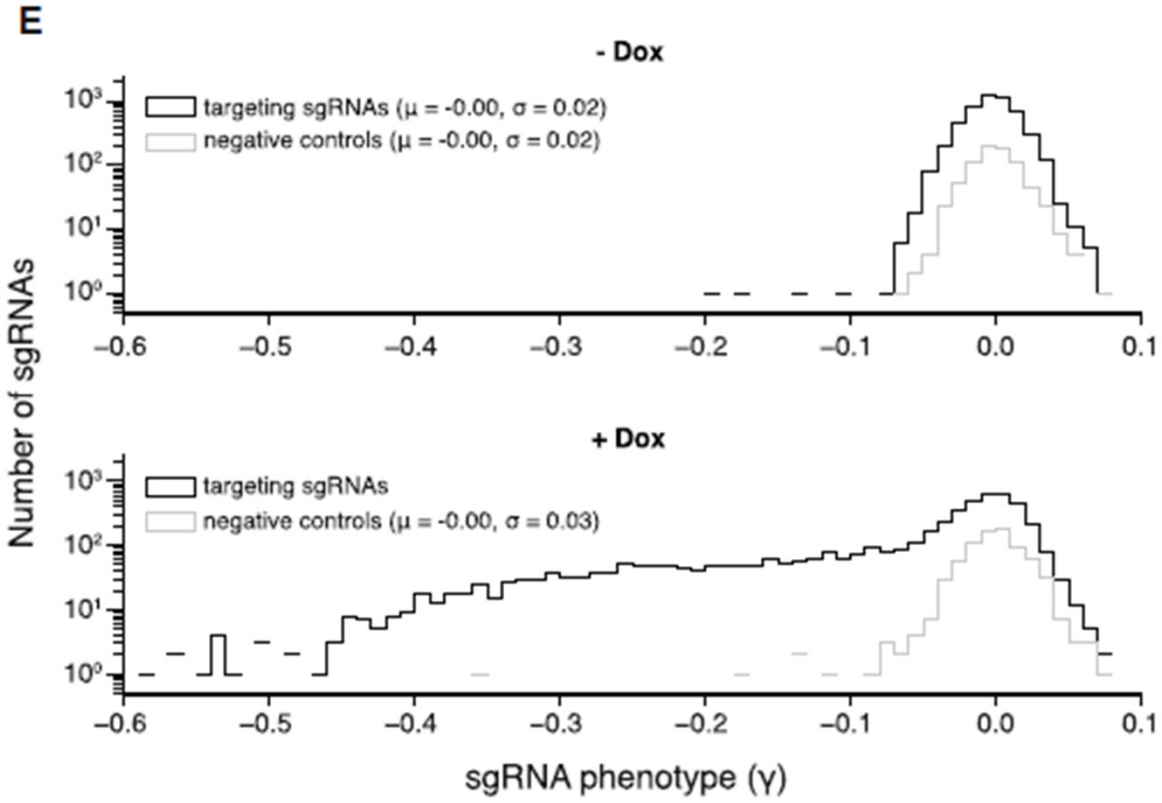



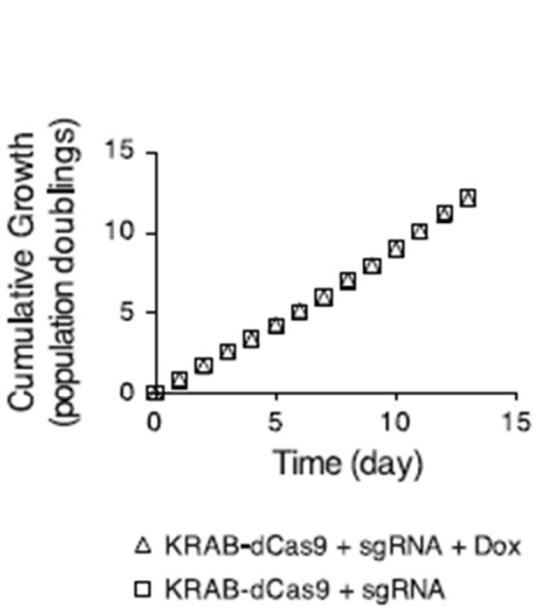

Complementary information can be obtained by loss- and gain-of-function genetic screenns and it highlights the utility of the platform for future studies into tumor biology and cell differentiation




KRAB-dCas9 does not create a permanently repressive chromatin state at targeted promoters

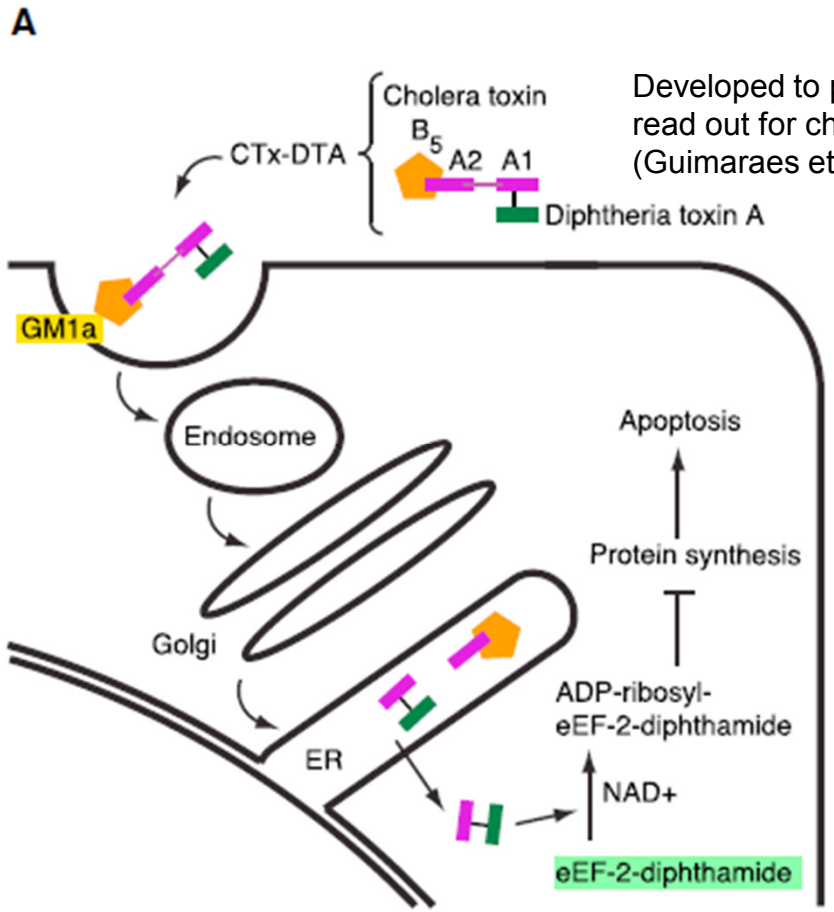
**Sublibrary**: targeting 426 genes (10/TSS = 4,923sgRNAs) with 750 nontargeting control sgRNAs.



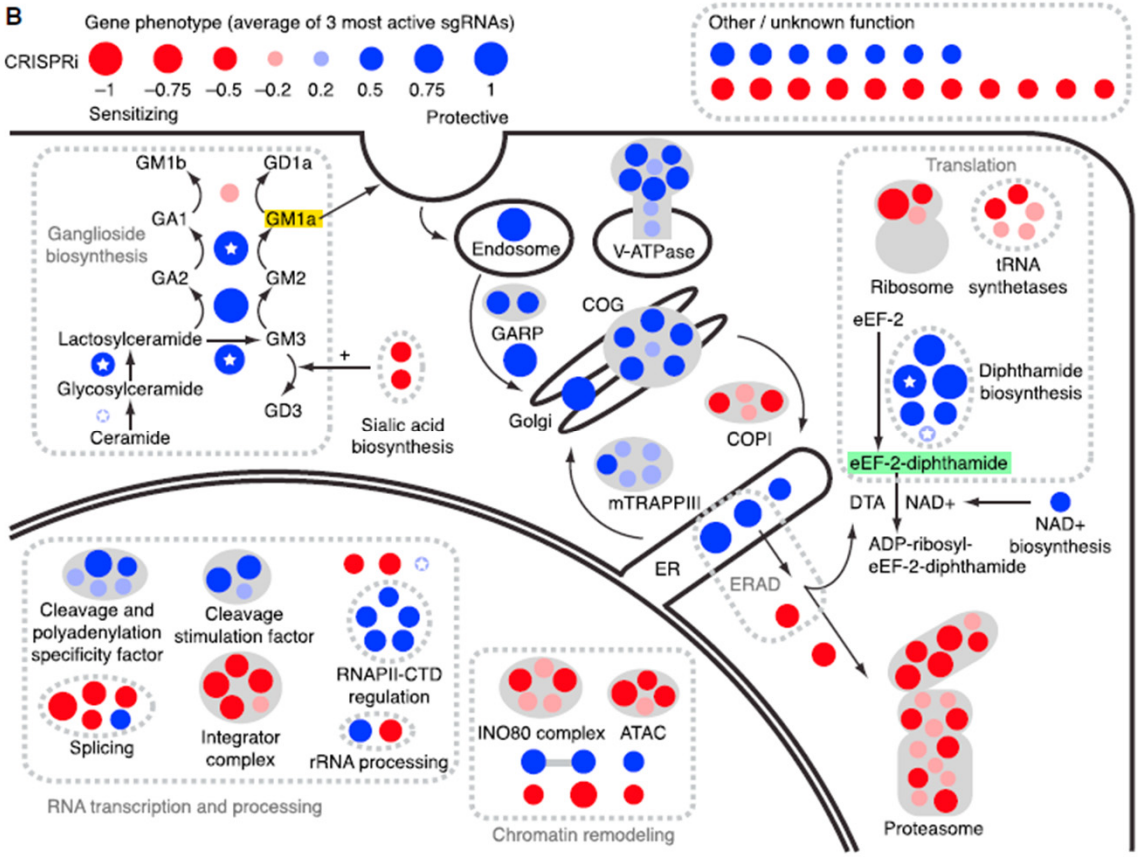

CRISPRi is nontoxic, inducible and reversible

# A genome-scale CRISPRi screen reveals pathways and complexes that govern response to cholera and diphteria toxin




Developed to provide a growth read out for cholera intoxication (Guimaraes et al., 2011)

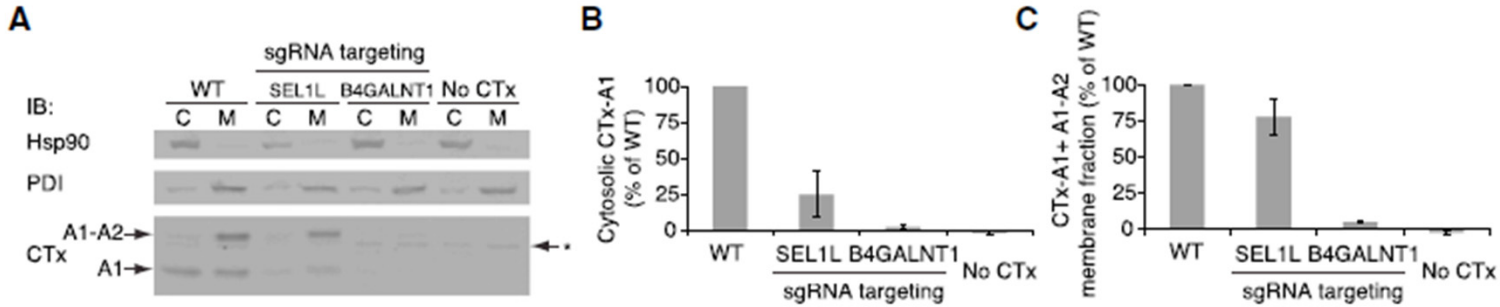
K562 cells expressing the CRISPRi library and dCas9-KRAB
+/- several pulses of CTx-DTA over 10 days



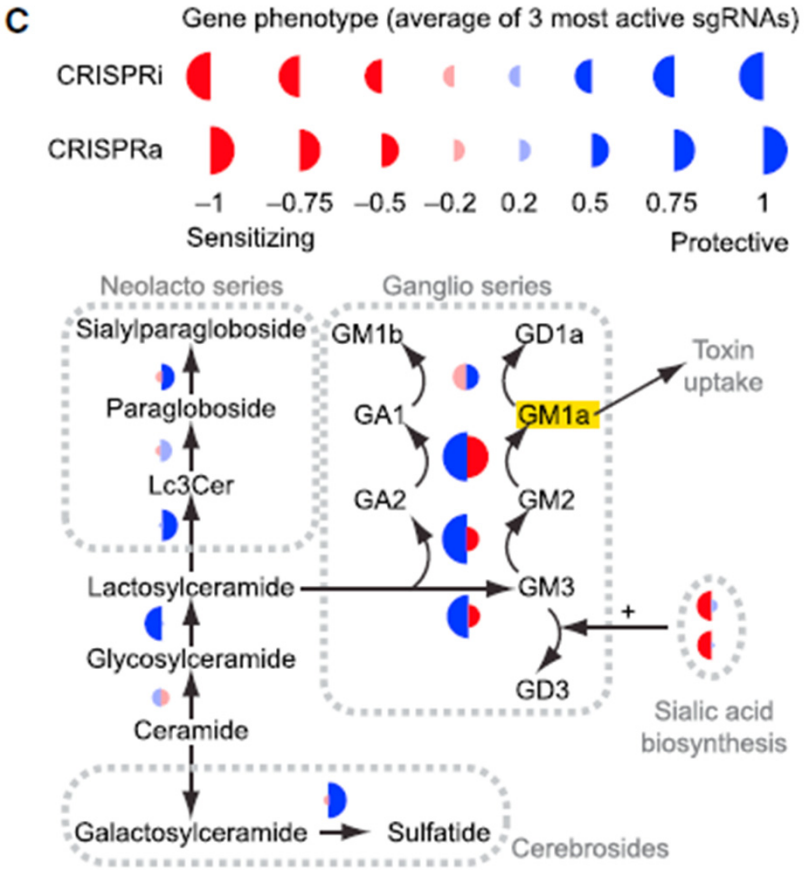

It illustrates the value of being able to detect sensitizing and protective gens to dissect biological functions

They identified complexes and pathways that had not been linked to cholera toxin biology yet -> highlighting the potential of CRISPRi as a discovery platform

Retrotranslocation of the catalytic chain of CTx has been proposed to be mediated by the ER-associated degradation (ERAD) pathway



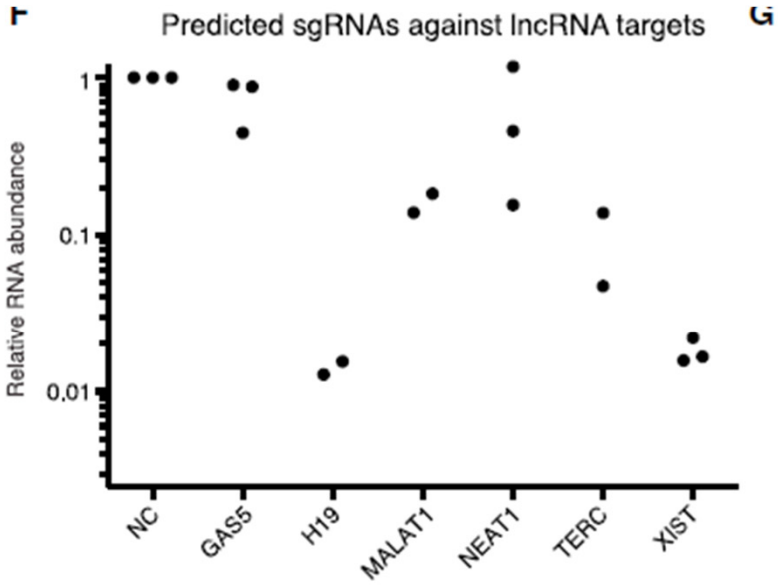
# Genome scale CRISPRa screen of Cholera-Diphteria Toxin complements and extends CRISPRi results




CRISPRa reveald additional and complementary information

Results highlight the capacity of CRISPRa to complement CRISPRi by querying the consequences of upregulating pathways that may otherwise be inactive

# Knockdown of noncoding RNAs

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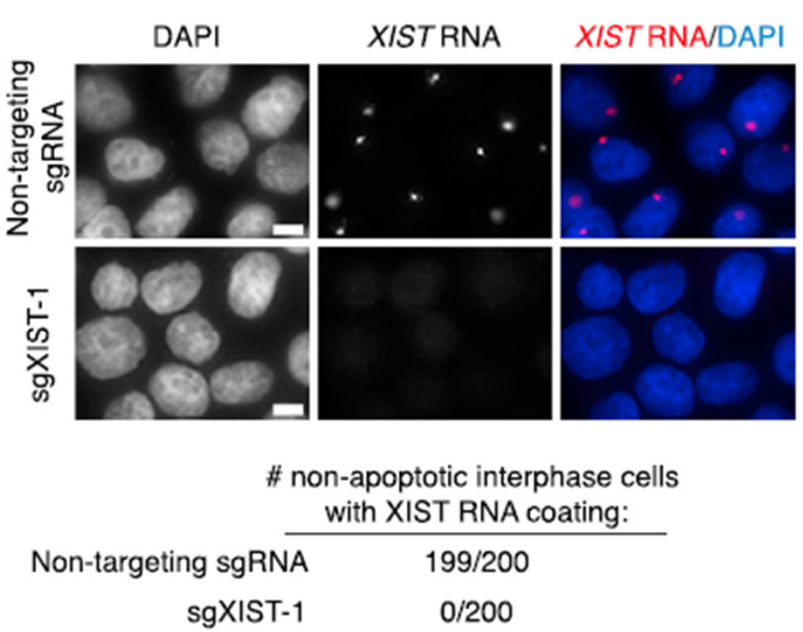


CRISPRi can effectively repress lncRNA expression, enabling future systematic studies of noncoding gene function

# Summary

- Established CRISPRi/a as robust tools for manipulating transcription of endogenous genes
- Demonstrated that it can be used to screen for loss of function and gain of function phenotypes in a pooled format
- Identified known and unknown genes that control growth of K562 cells or that modulate sensitivity to a toxin

Key feature of CRISPRi/a: low incidence of off-target effects that simplifies validation and interpretation of screening results

- CRISPRi/a complexes bound outside a narrow window around the TSS largely fail to modulate transcription
- CRISPRi activity is highly sensitive to mismatches between the sgRNA and target DNA