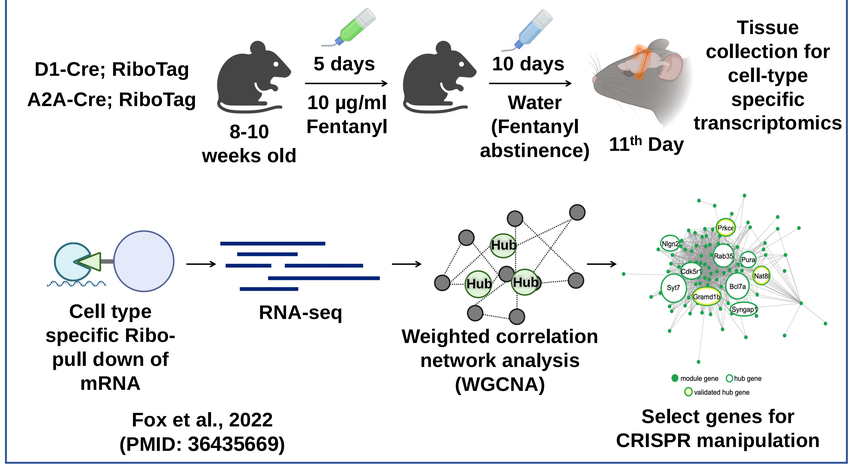
CasMINI toolkit for cell-type specific and light-inducible epigenome editing applications

Mikah Green[1], *Shanmugasundaram Ganapathy*[1], *Ramesh Chandra*[1,2], *Nikhil Hajirnis*[1], *Eric Choi*[1], *Mary Kay Lobo*[1]

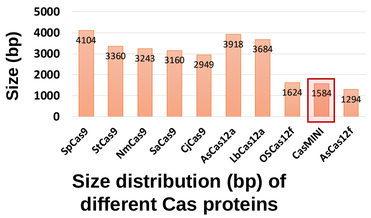
[1]*University of Maryland School of Medicine, Baltimore MD,* [2]*Virus Vector Core, University of Maryland, Baltimore MD*

Background



Fox et al., 2022
(PMID: 36435669)

dCasMINI Design

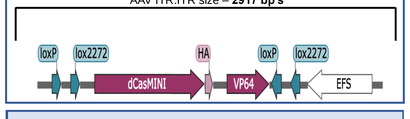


Size distribution (bp) of different Cas proteins

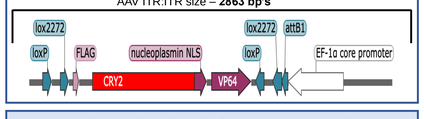
dCasMINI-CRISPRa
CRISPR activation

dCasMINI-CRISPRi
CRISPR interference

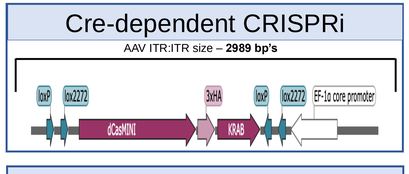
Cre-dependent CRISPRa



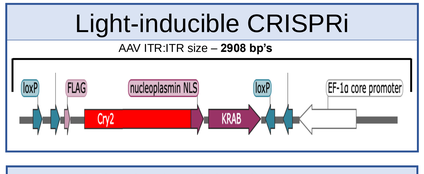
Light-inducible CRISPRa



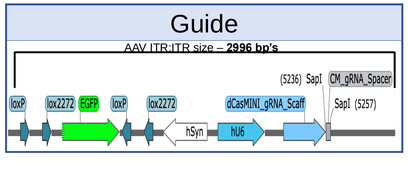
Cre-dependent CRISPRi



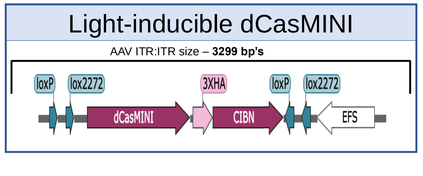
Light-inducible CRISPRi



Guide



Light-inducible dCasMINI



In-vitro validation of expression

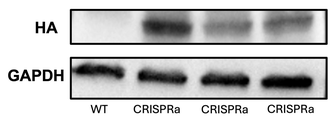


Figure 1. Western blot results confirming expression of Cre-dependent dCasMINI-VP64 & dCasMINI-KRAB vectors in Neuro2a cells. Cells were transfected using a liposomal transfection method and were harvested 48 hours post-transfection.

In-vitro cre-dependent dCasMINI

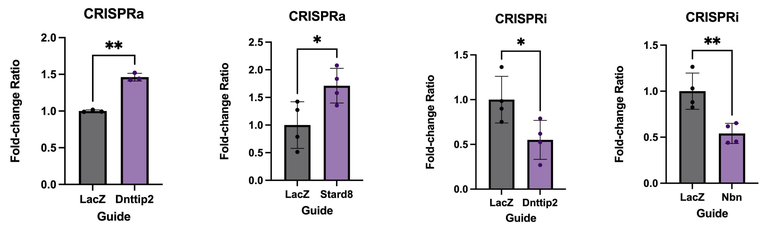


Figure 2. gRNA for target genes or LacZ were transfected along with CRISPRa/I constructs in Neuro2A cells (3-4 biological replicates per gRNA condition). Cells were then harvested and analyzed by RT-qPCR using GAPDH as our housekeeping gene. Welch's t-test, *p<0.05, **p<0.01.

In-vitro light-inducible dCasMINI

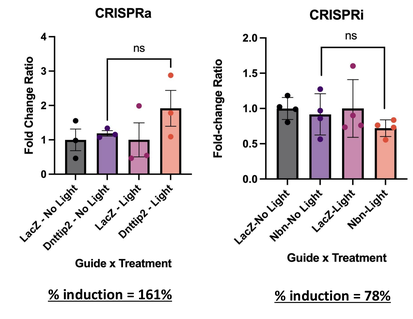


Figure 3. Neuro2a cells were transfected with gRNA for target gene or LacZ along with CIBN-dCasMINI and either Cry2-VP64 or Cry2-KRAB (6-8 biological replicates per gRNA condition). 48 hours after transfection, DMEM was replaced with photostable media for 3hrs. After 3hrs, no light control cells were harvested while light treated samples received 2 hours of 1mW light at 470nm, followed by 1 hour with no light before being harvested. Cells were then analyzed by RT-qPCR using GAPDH as our housekeeping gene. Welch's t-test, *p<0.05.

In-vivo validation of expression

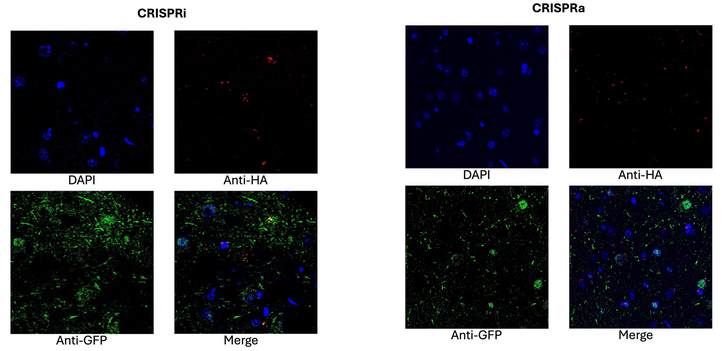


Figure 4. Immunohistochemistry of A2A-Cre mouse with AAV-dCasMINI-HA-VP64 + AAV-LacZ-gRNA injected in the nucleus accumbens (NAc). Also, D1-Cre mouse with AAV-dCasMINI-HA-KRAB + AAV-LacZ-gRNA injected in the NAc. Images were taken using 63x magnification on a confocal microscope.

Conclusion

- Successful generation of dCasMINI CRISPR epigenome editing constructs with validation of expression in-vitro
- Successful upregulation/downregulation of target hub genes using cre-inducible CRISPRa/i tools in-vitro
- Successful upregulation/downregulation of target hub genes using light-inducible CRISPRa/i tools in-vitro
- Successful expression of dCasMINI CRISPRa/i & gRNA constructs in-vivo

Future Directions

- Further validation of dCasMINI virus/plasmid expression in mouse NAc
- Test gRNA efficiency in-vivo with both CRISPRa/i
- Test effect of hub gene manipulation on expression of other hub genes within the same module
- Validate opto-dCasMINI expression in-vivo
- Use optogenetic fiber implant to stimulate gene expression manipulation in-vivo using the light-inducible tools
- Examine effects of manipulating hub gene expression in the Nac with the CRISPRa/i tools on fentanyl intake and fentanyl related behaviors

Funding & Contact Info

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LinkedIn

Mikah.green@som.umaryland.edu