

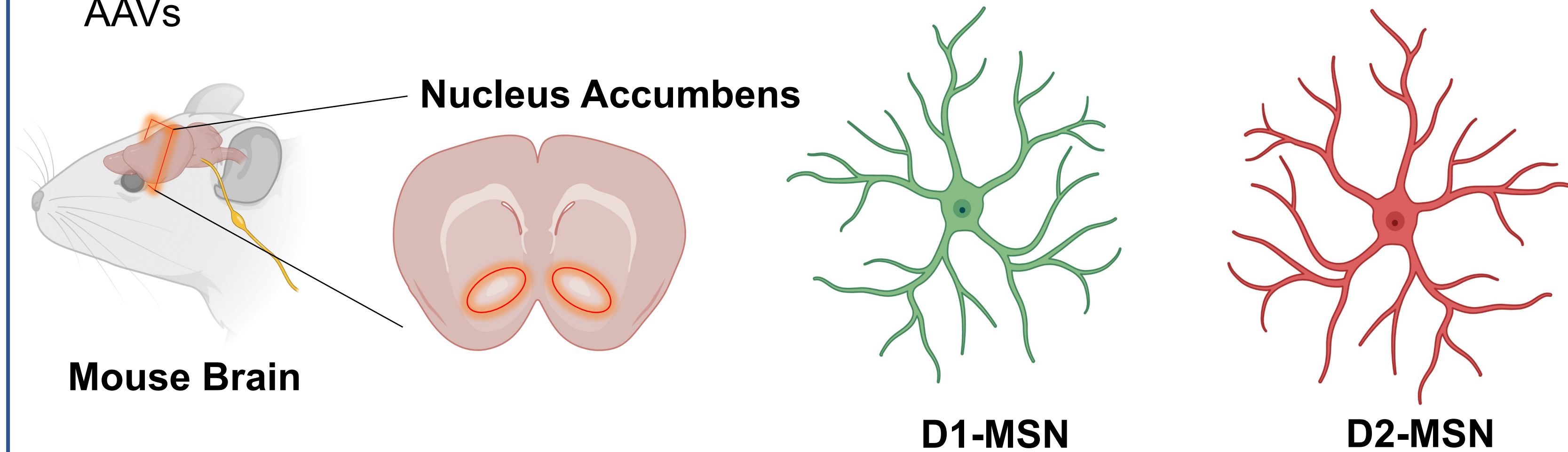
Small but Mighty: CRISPR Epigenome Editing Using a Cas12f-derived System for Gene Expression Interference of Fentanyl Regulated Genes

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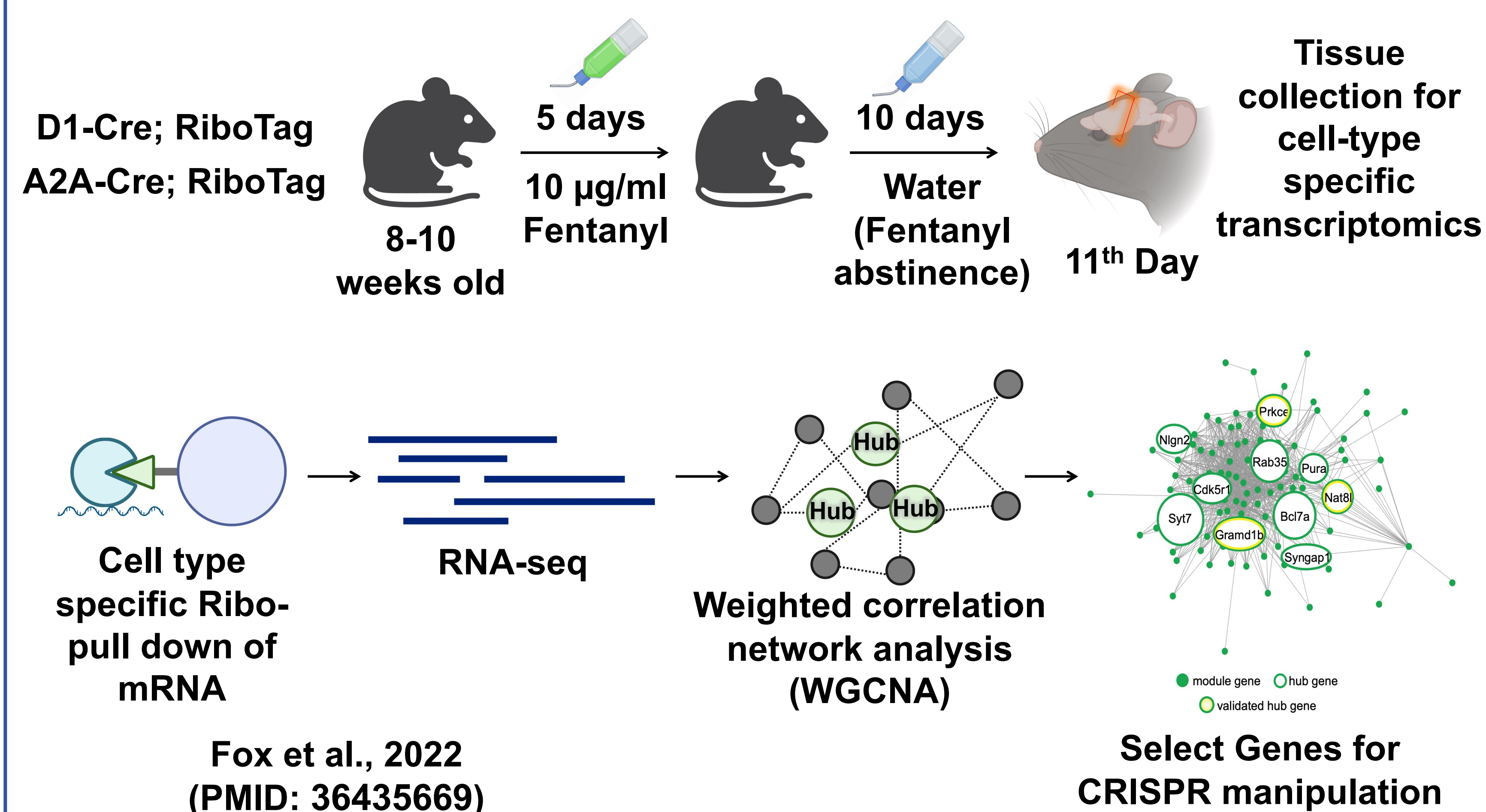
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Introduction

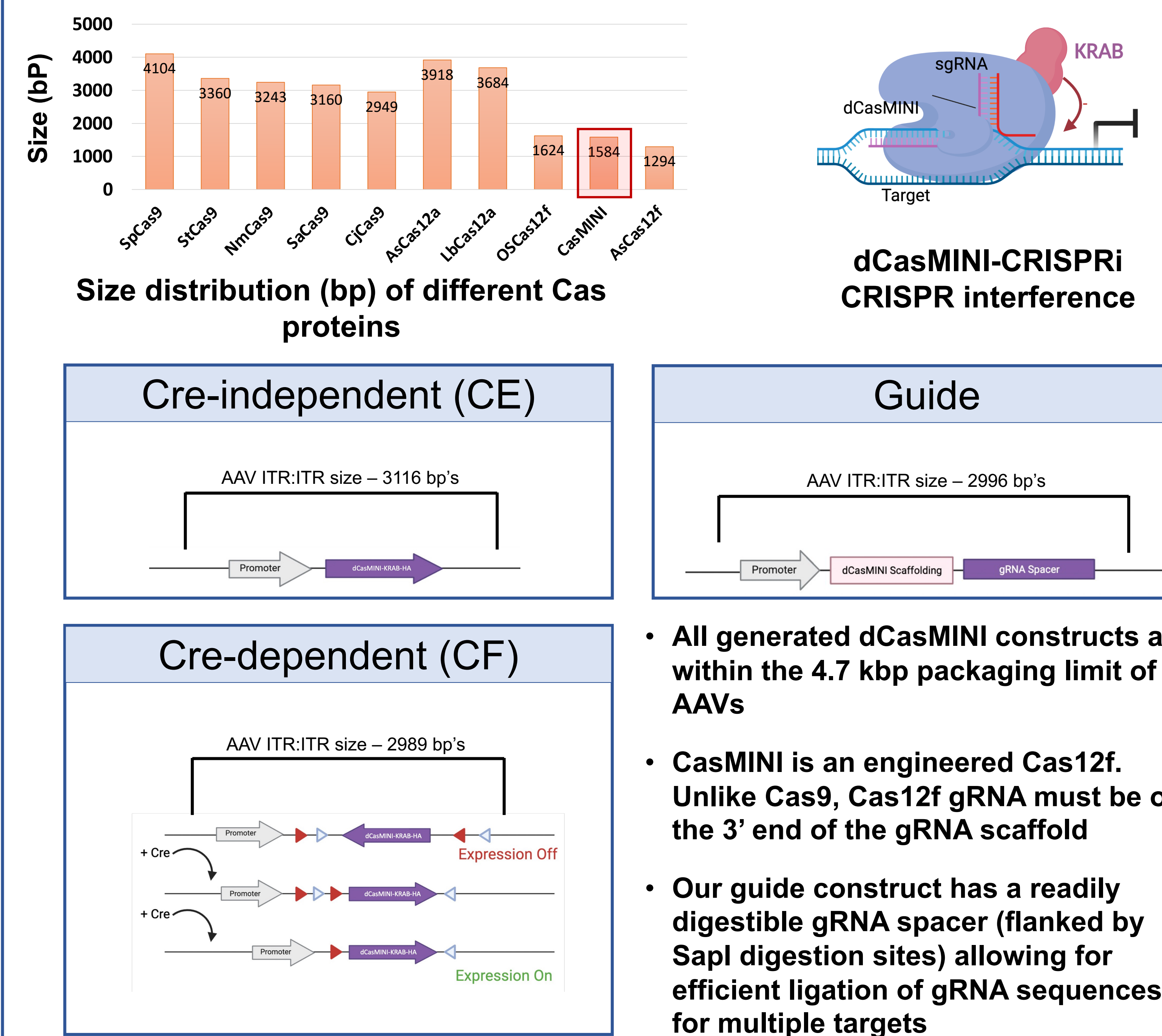
- Fentanyl is a synthetic opioid that is 50 times stronger than heroin. There were over 70,000 deaths involving synthetic opioids (primarily fentanyl) in 2021.
- The nucleus accumbens (NAc) is a brain region heavily involved in reward circuitry and it is significantly impacted by addictive drugs, including fentanyl.
- Within the NAc, the majority of neurons are medium spiny neurons (MSNs), with two distinct populations - dopamine receptor 1 (D1) enriched MSNs and dopamine receptor 2 (D2) enriched MSNs.
- Cell-type specific transcriptomic analysis of D1- and D2-MSNs was conducted by our lab using NAc tissue from mice that underwent abstinence following exposure to fentanyl in drinking water.
- Weighted gene correlation network analysis (WGCNA) provided an unbiased view into fentanyl abstinence-induced changes to the transcriptional landscape of these two populations of MSNs in the NAc.
- The present work is dedicated to developing a tool that can epigenetically downregulate target hub genes that were differentially enriched in the two MSN populations following abstinence from fentanyl.
- Here, we are developing a CRISPR interference (CRISPRi) tool using the recently engineered dCasMINI platform due to its small size and ability to be packaged into AAVs



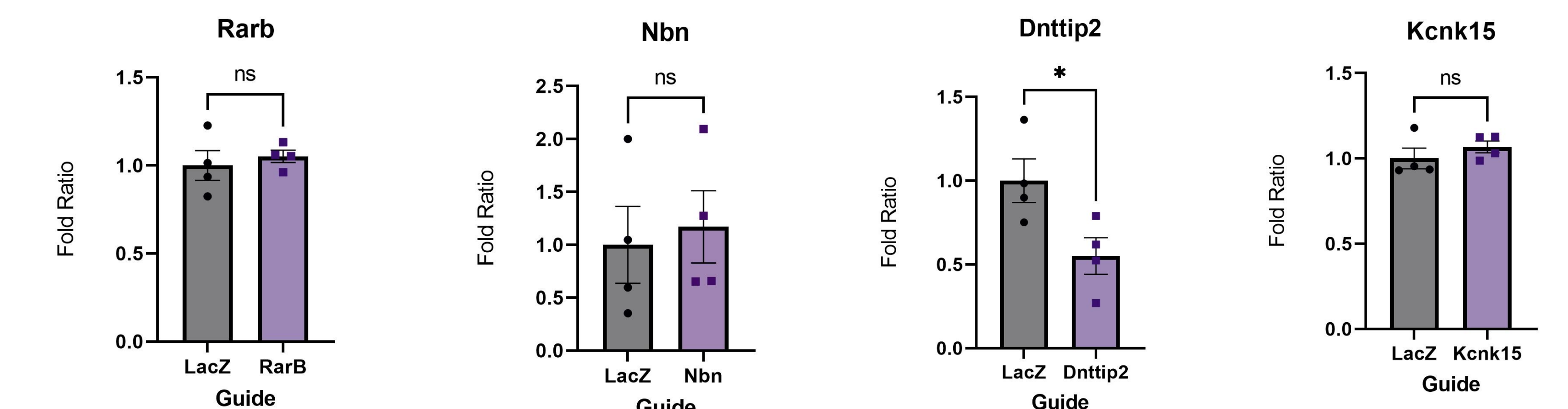
Background



dCasMINI CRISPRi Design



In-Vitro Testing of dCasMINI-CRISPRi



Conclusion

- CasMINI, specifically dCasMINI-KRAB, successfully expresses in neuronal cells in vitro, which has not been previously demonstrated
- dCasMINI-KRAB can efficiently downregulate target gene expression levels in Neuro2a cells.
- Even though we did not observe downregulation of several of our targets using our dCasMINI-KRAB platform, it does not necessarily mean that this system won't work for those targets without further gRNA optimization.

Future Directions

- Generate gRNA constructs with different sequences for targets that we did not observe downregulation in, gRNA sequences used come from -500 to 0 bp's from the TSS and were chosen based off the smallest number of off-target effects in the *mus musculus* genome.
- Transfect mouse NAc with our CRISPRi + gRNA vectors after packaging in AAVs to validate efficacy of the dCasMINI platform in vivo.
- Assess fentanyl regulated MSN-subtype gene networks after dCasMINI CRISPR epigenome editing of hub genes.
- Assess stress vulnerability, fentanyl intake and relapse after dCasMINI CRISPR epigenome editing of MSN subtype hub genes.

In-Vitro Validation of Expression

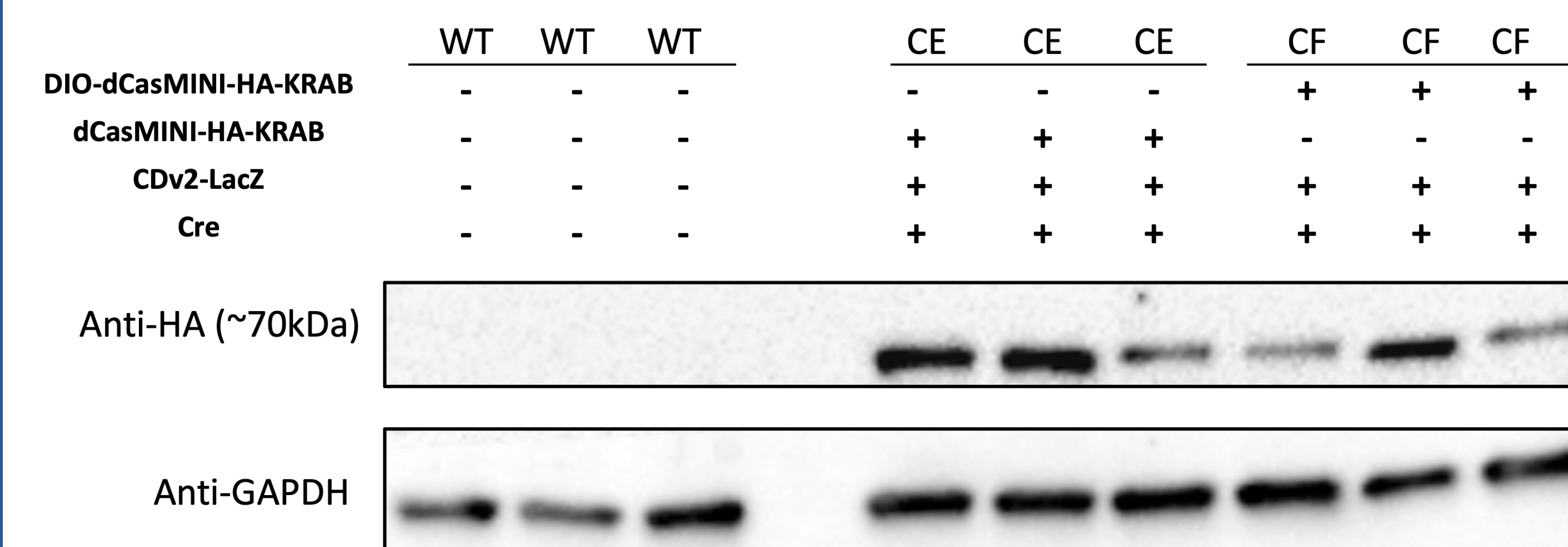


Figure 1. Western blot results confirming expression of Cre-dependent and Cre-independent dCasMINI-KRAB vectors in Neuro2a cells. Three biological replicates per condition using a liposomal transfection method, cells were harvested 48 hours post-transfection.

For the first time we have confirmed expression of a CasMINI construct in neuronal cells, providing the foundation for further application of this platform in the brain.

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