Optimizing CRISPR/Cas9-Mediated *Bax* Gene Knock-Out in Sympathetic Neurons

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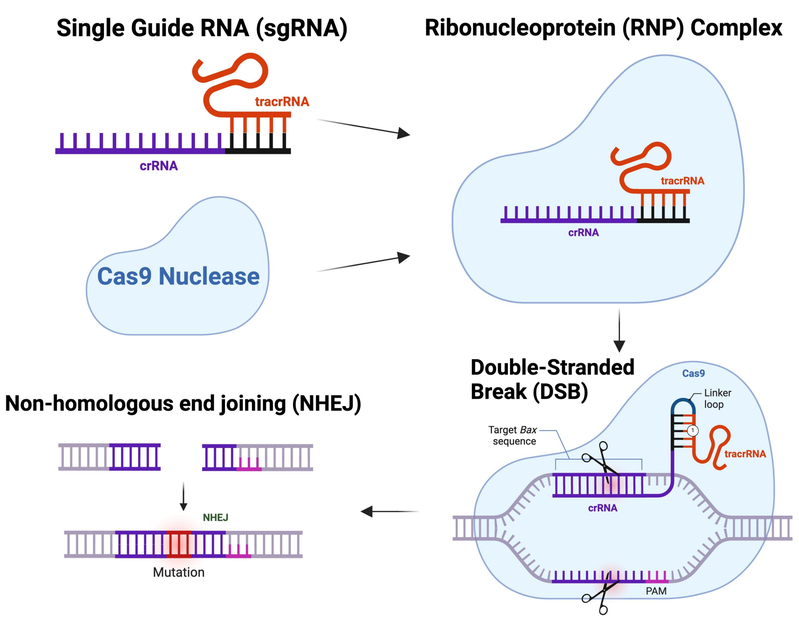
Introduction

- CRISPR-Cas9 technology enables precise genomic insertions or deletions
- CRISPR requires a Cas9 nuclease and target-specific single guide (sgRNA)
- Electroporation of sympathetic neurons yields low efficiency however lentiviral transduction offers a viable method for the delivery of genetic material
- Establishing an effective CRISPR-Cas9 system in sympathetic neurons remains a challenge

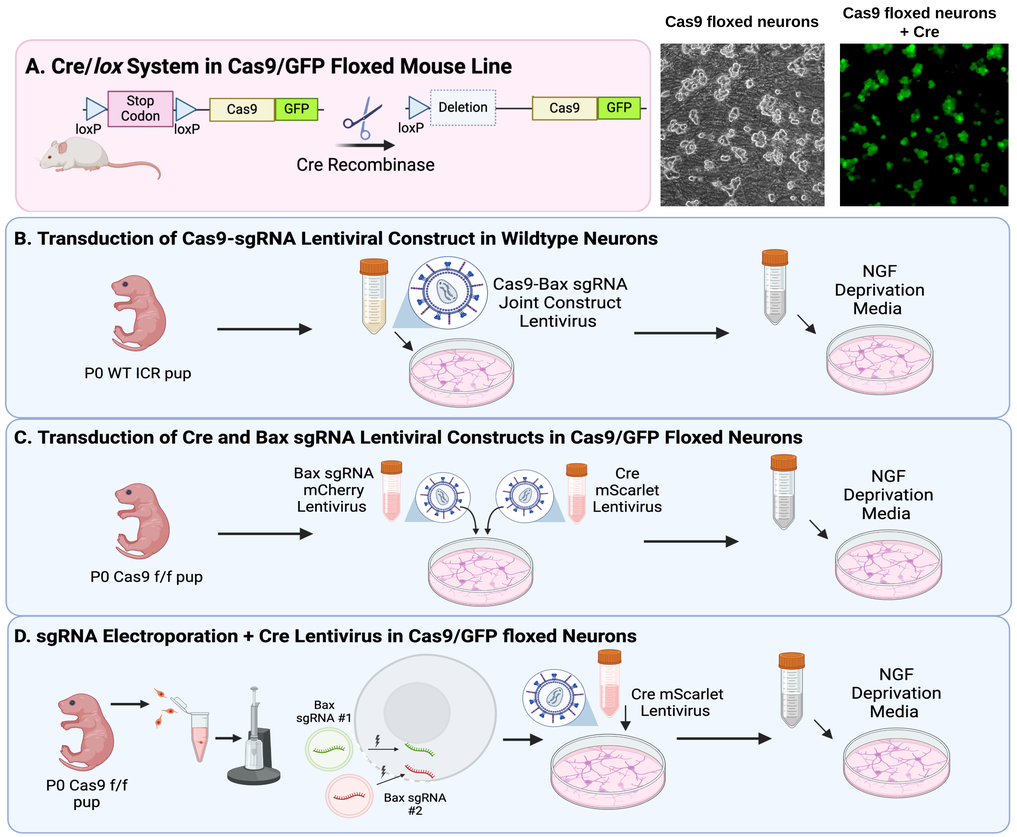
Objective

- We aim to assess and compare the knockout efficacy of three CRISPR/Cas9-mediated approaches on the apoptotic regulator *Bax* gene in sympathetic neurons.
- **We hypothesize that a lentiviral approach will yield the most efficient CRISPR-Cas9 *Bax* gene knockout in neurons.**

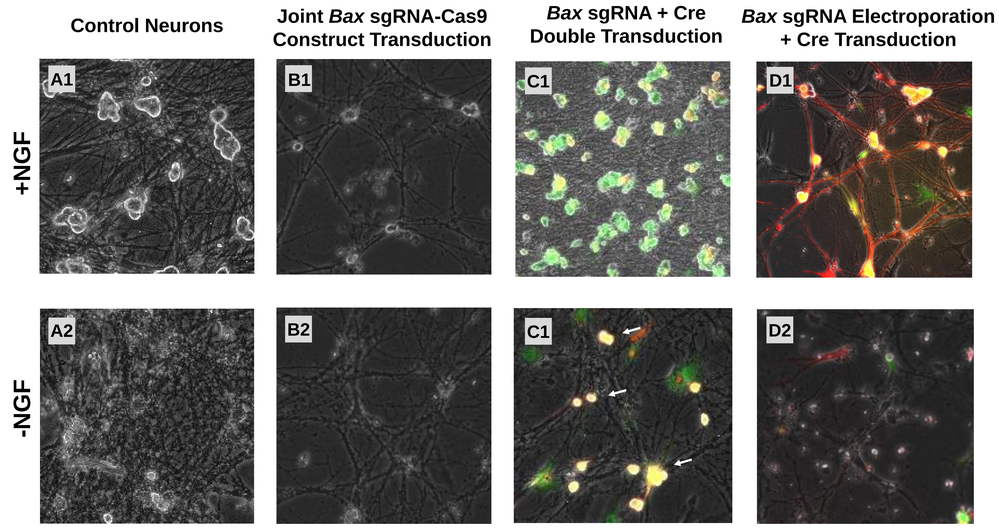
CRISPR-Cas9 Mediated *Bax* Knockout



Experimental Approach



Results



A: Control Cas9 f/f neurons pre-deprivation and 24 hours post-deprivation.
B: Wildtype neurons transduced with a joint *Bax* sgRNA-Cas9 construct (no fluorescent marker).
C: Cas9 f/f neurons transduced with both *Bax* sgRNA lentivirus and Cre lentivirus. (White arrows: double-transduced, alive neurons post NGF deprivation.)
D: Cas9 f/f neurons electroporated with *Bax* sgRNA (no fluorescent marker) and transduced with Cre (mScarlet) lentivirus.

Conclusion

- Lentiviral transduction of *Bax* sgRNA and Cre recombinase in Cas9 floxed neurons is more effective at knocking-out *Bax* than both electroporation of sgRNA in Cas9 f/f neurons and transduction of a single sgRNA-Cas9 lentiviral construct.

Future Directions

- Evaluate the efficacy of transducing two lentiviruses in wild-type neurons: *Bax* sgRNA and Cas9 nuclease.
- Use the CRISPR-Cas9 system to investigate neuronal apoptosis and axon pruning by targeting genes along these molecular pathways.
- Target genes within the BH3-only protein family, elucidating their individual contribution to the rate of neuronal apoptosis.

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