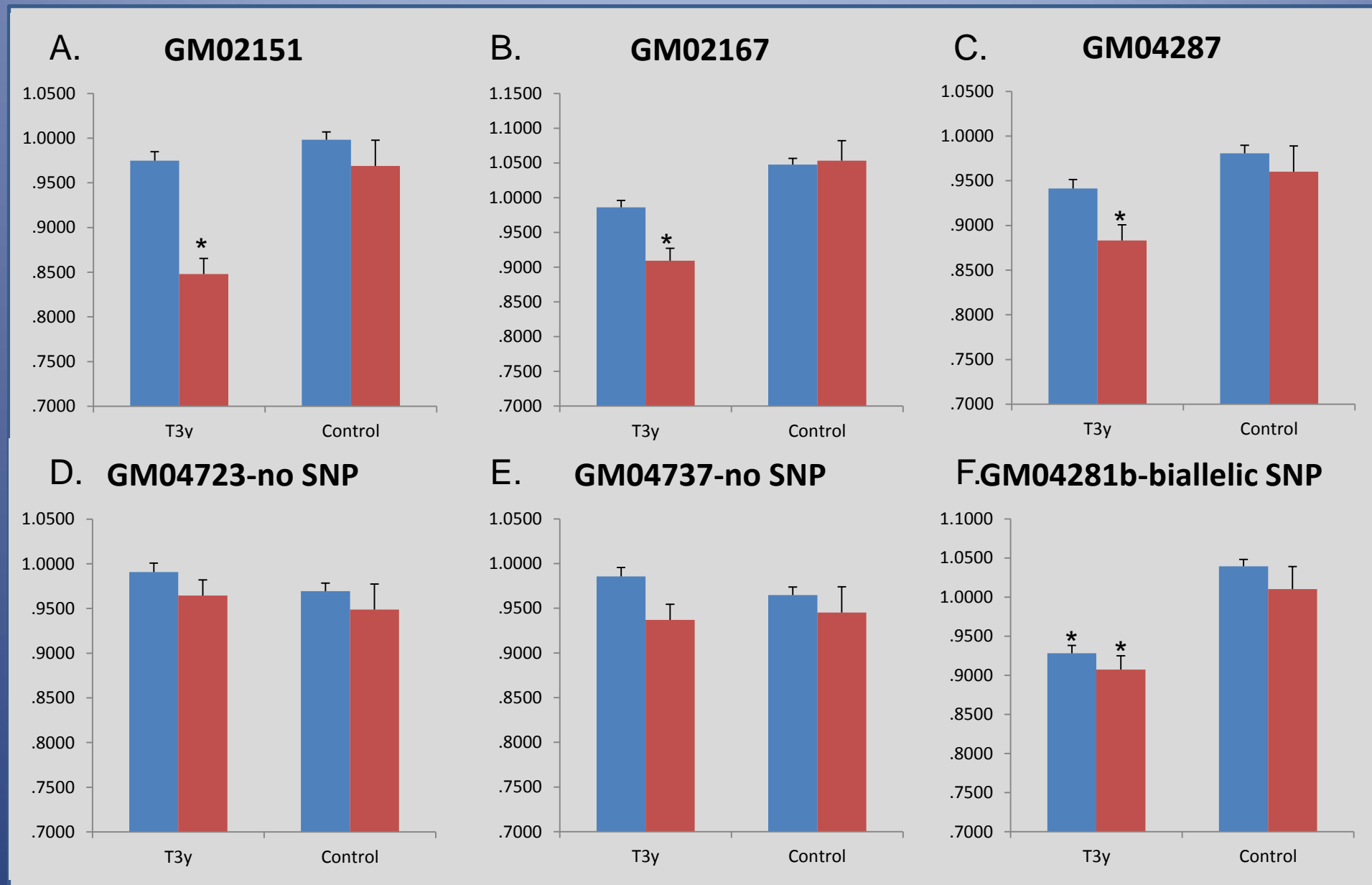


# Targeted Epigenetic Modification

Kyle Fink, PhD – UC Davis Stem Cell Program  
Adjunct Assistant Professor, Department of Neurology

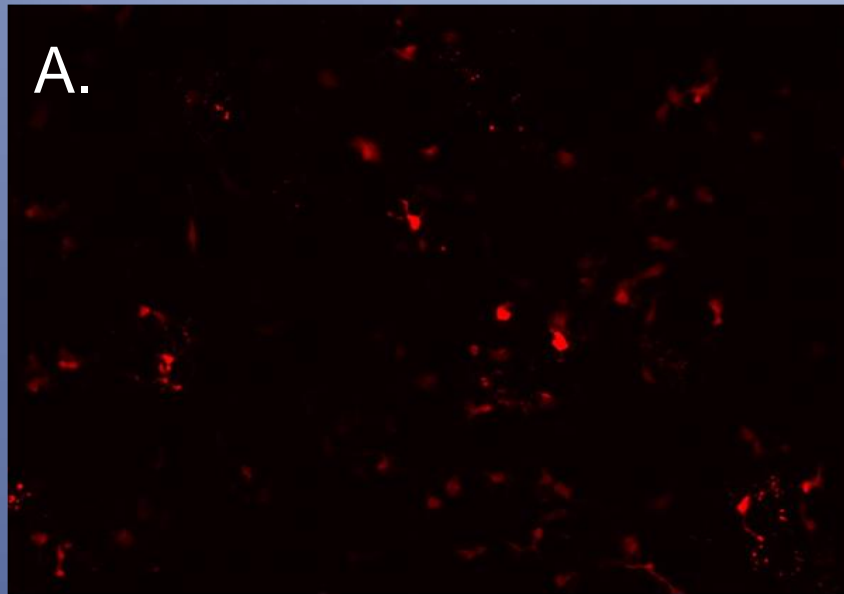
- TALE
  - Derived from plant pathogenic bacteria from the genus *Xanthomas*
  - One of many DNA-targeting proteins
  - Each repeat comprises 33-35 amino acids.
  - Can be rapidly synthesized to target any base pair sequence
  - Highly efficient and specific with minimal off-target effects
- CRISPR/Cas9
  - Original Cas9 derived from *Streptococcus pyogenes*
  - Uses a synthetic guide RNA (gRNA) to deliver the Cas9 to a desired location
  - Cas9 can be used interchangeably with different gRNA allowing for rapid targeting and flexibility
- Both systems can be constructed with a variety of transcription factors to epigenetically regulate gene expression (i.e., nucleases, activators, repressors)

# Transcription Activator-like Effector (Graphic Summary)

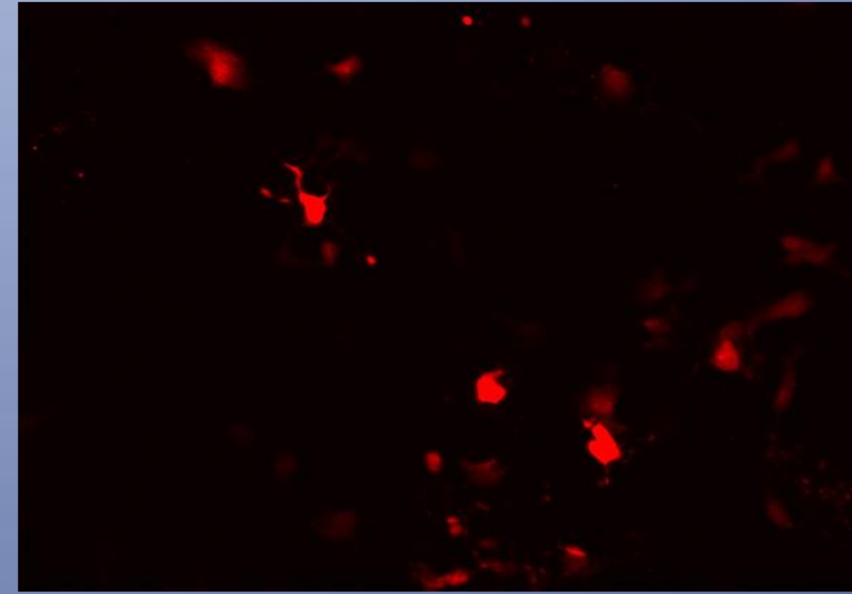


Human HD fibroblasts confirmed to have SNP rs3857369 display significant allele-specific reduction of the mutant allele (red bar) without significantly altering healthy allele expression (blue bar).

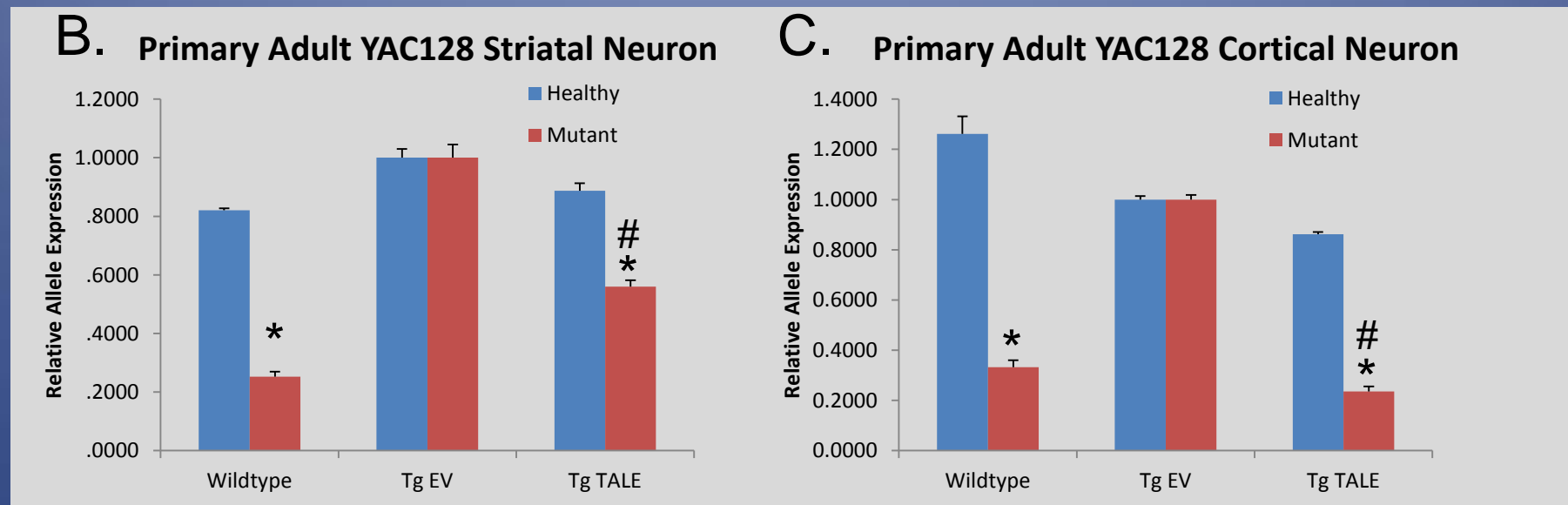
# Transcription Activator-like Effector (cont.)



T3y mCherry Treated YAC128 MSN Culture-10x

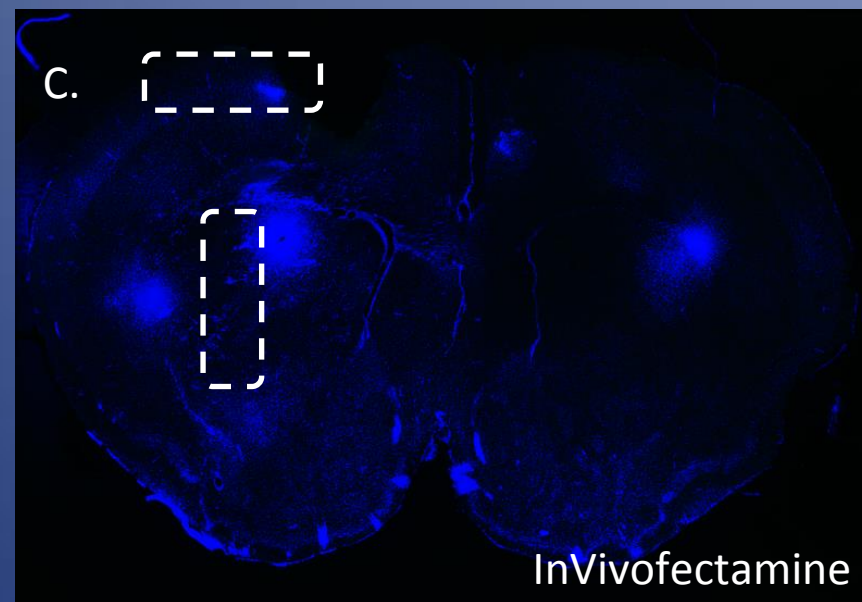
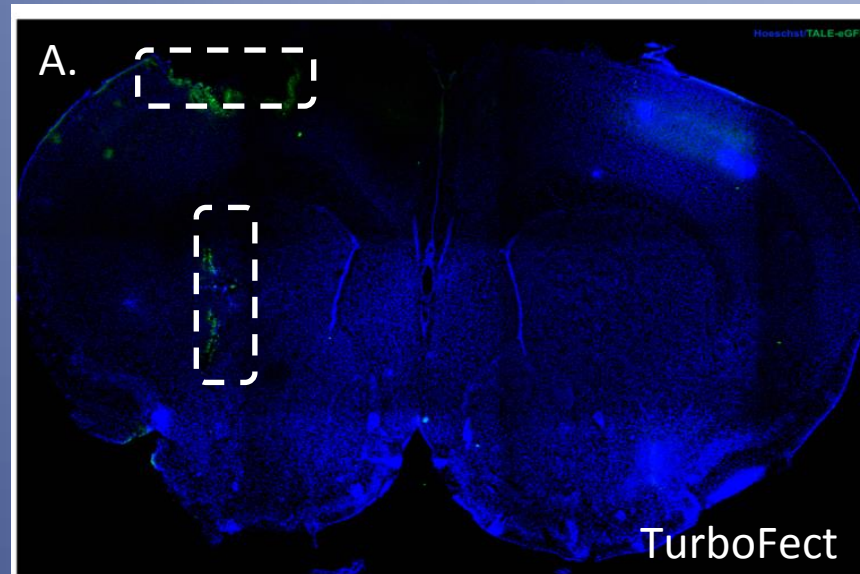


T3y mCherry Treated YAC128 MSN Culture-20x

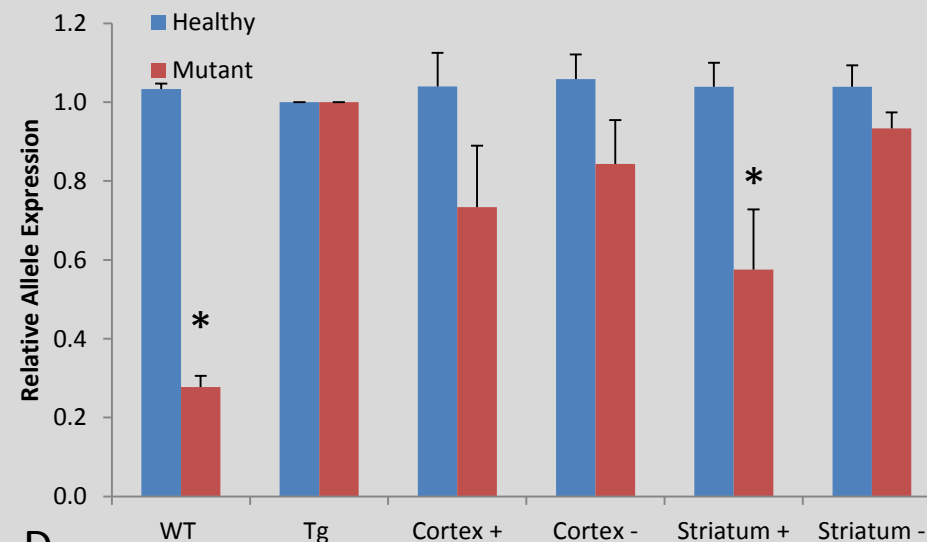


Primary cortical and striatal neurons cultured from a 10 month old YAC128 mouse treated with TALE T3y.

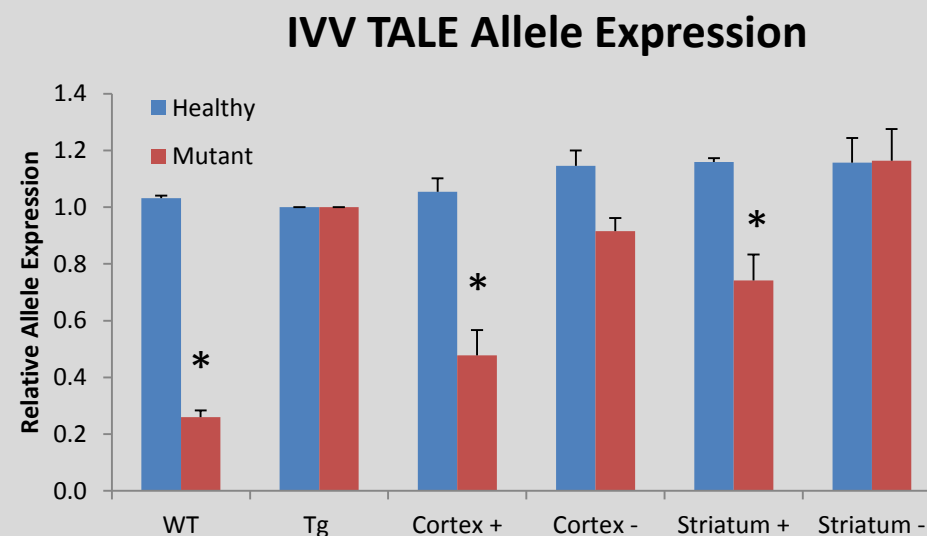
# Transcription Activator-like Effector (cont.)



## B. TurboTALE Allele Expression

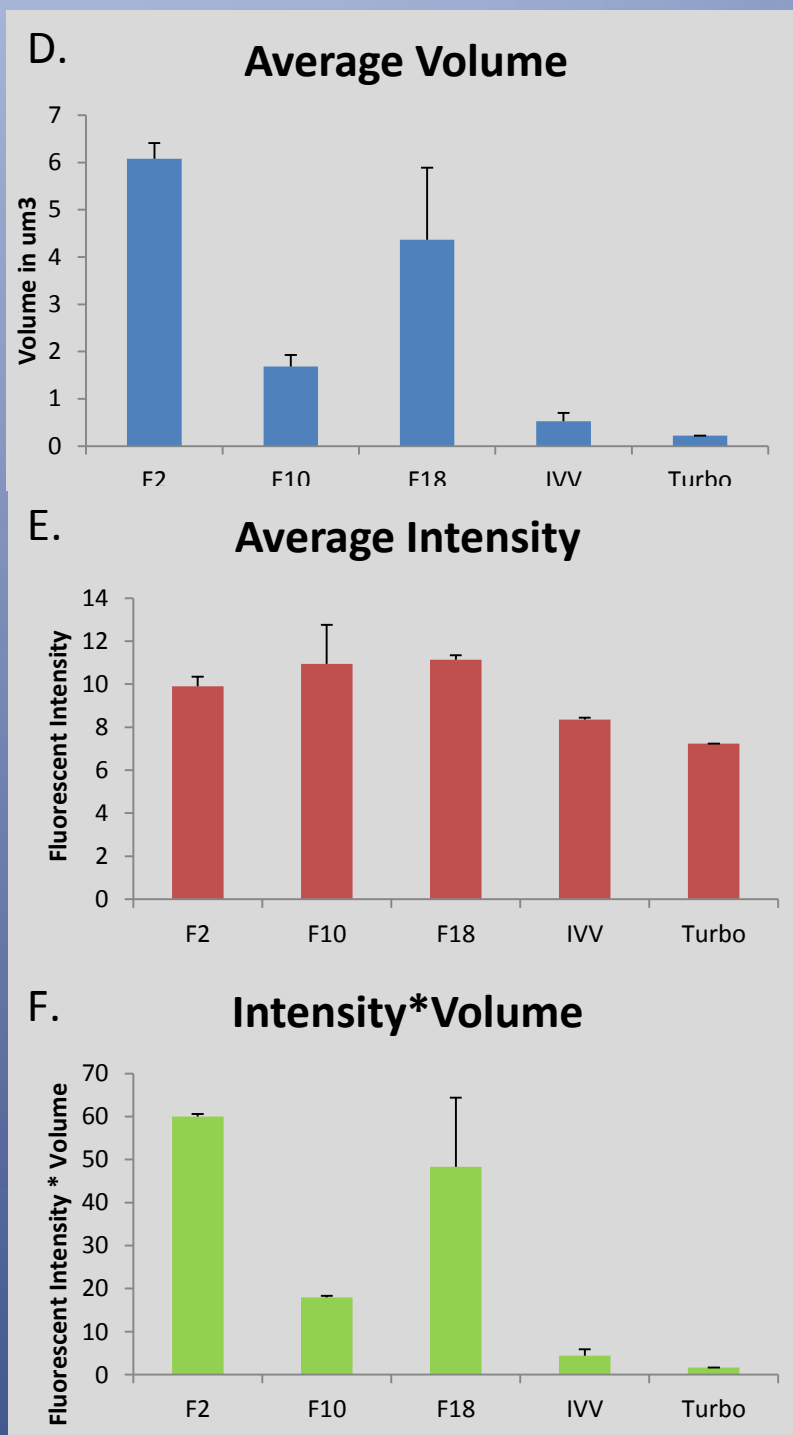
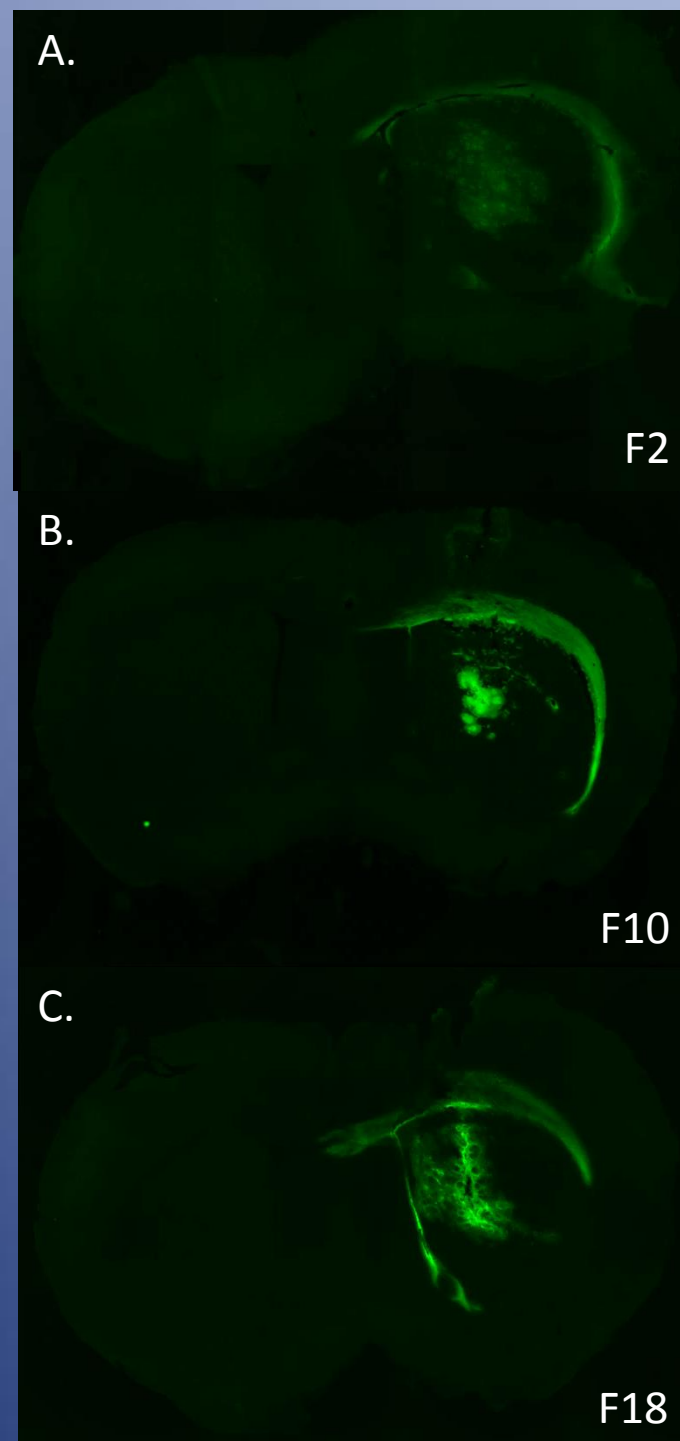


## D. IVV TALE Allele Expression



In vivo injection of the TALE plasmid and synthetic RNA using TurboFect and Invivofectamine.

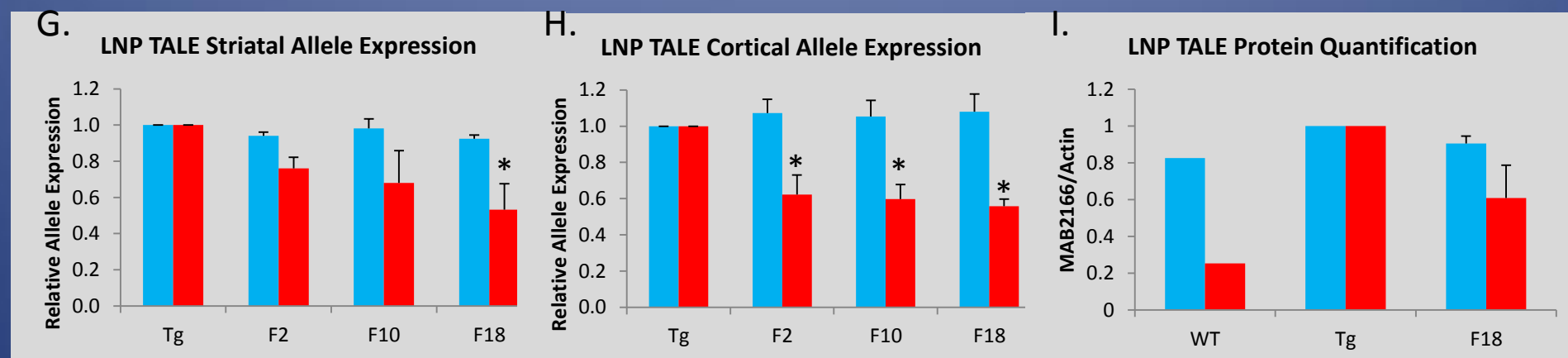
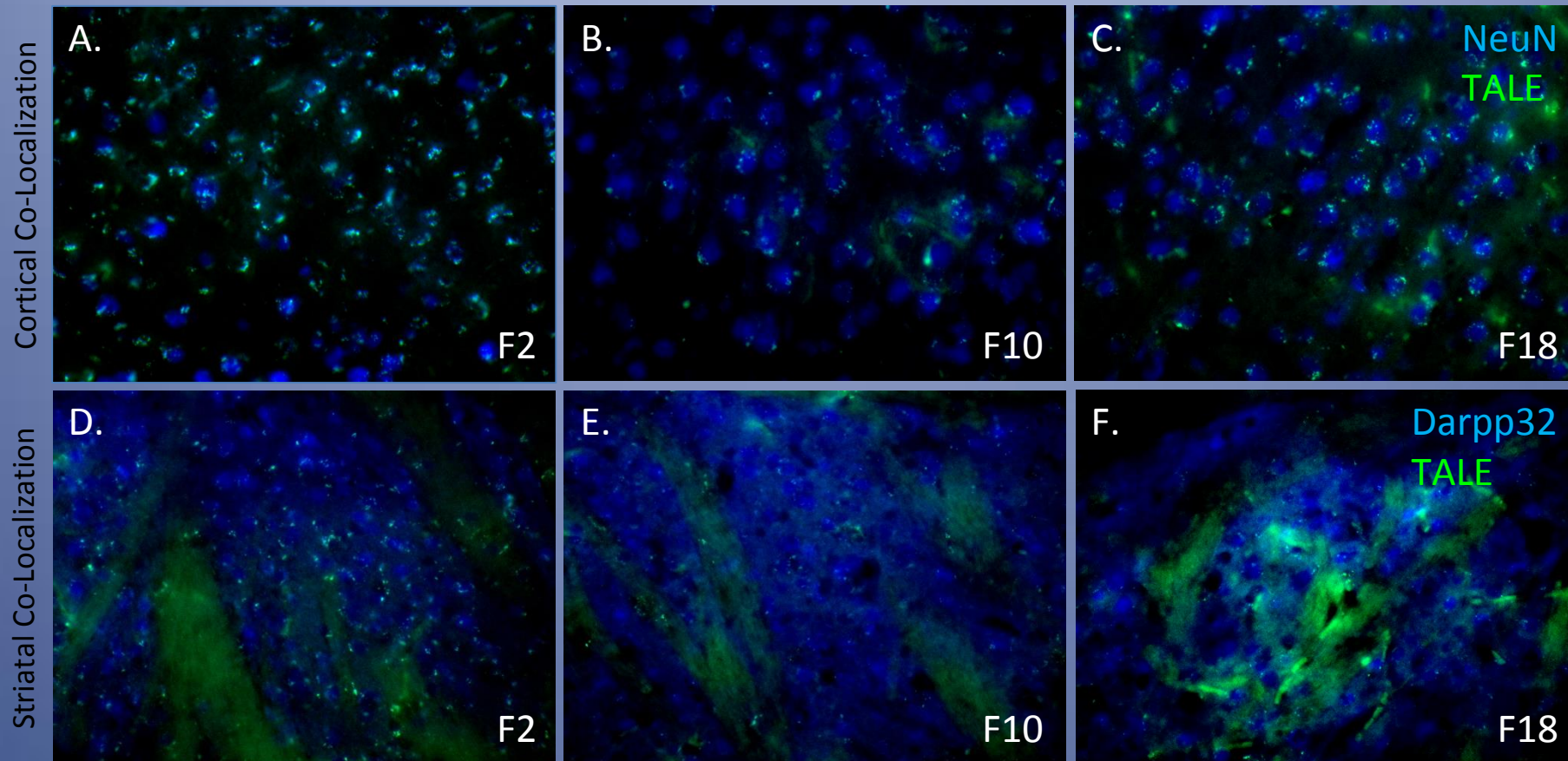
# Transcription Activator-like Effector (cont.)



Quantification of TALE biodistribution and expression following LNP encapsulation and unilateral injection.

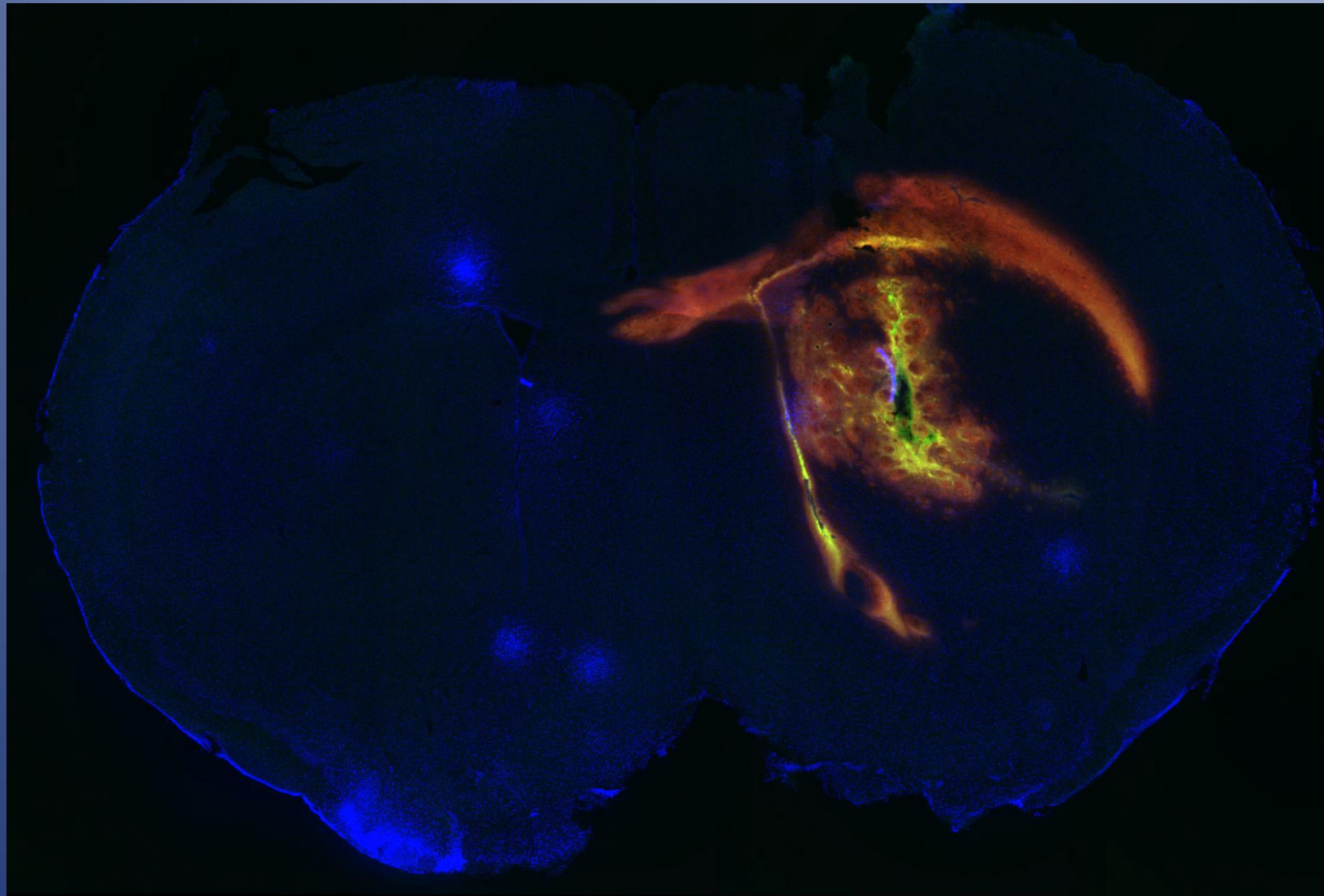
# Transcription Activator-like Effector (cont.)

Cortical and Striatal and Co-localization

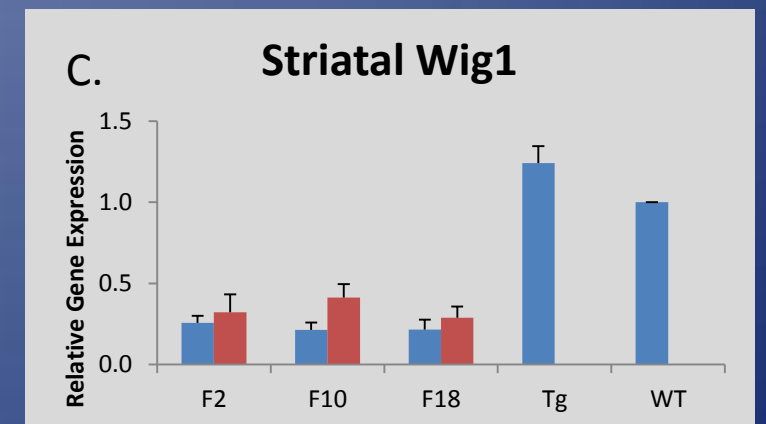
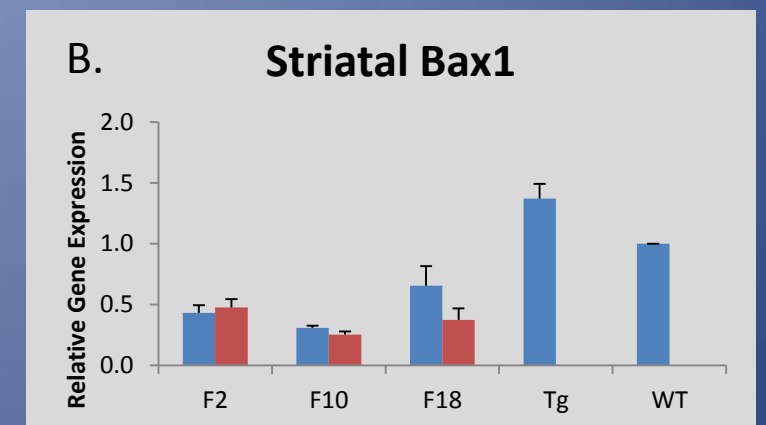
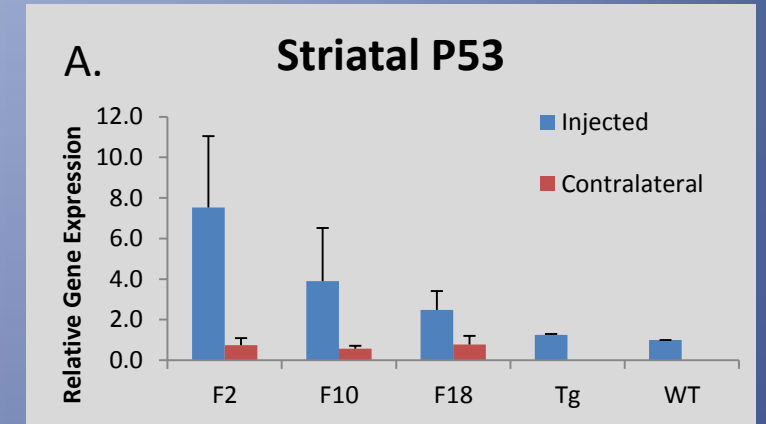


Co-localization of the TALE T3y with cortical and striatal neurons with TaqMan SNP genotyping for allele expression and Western Blot for protein quantification.

# Transcription Activator-like Effector (cont.)

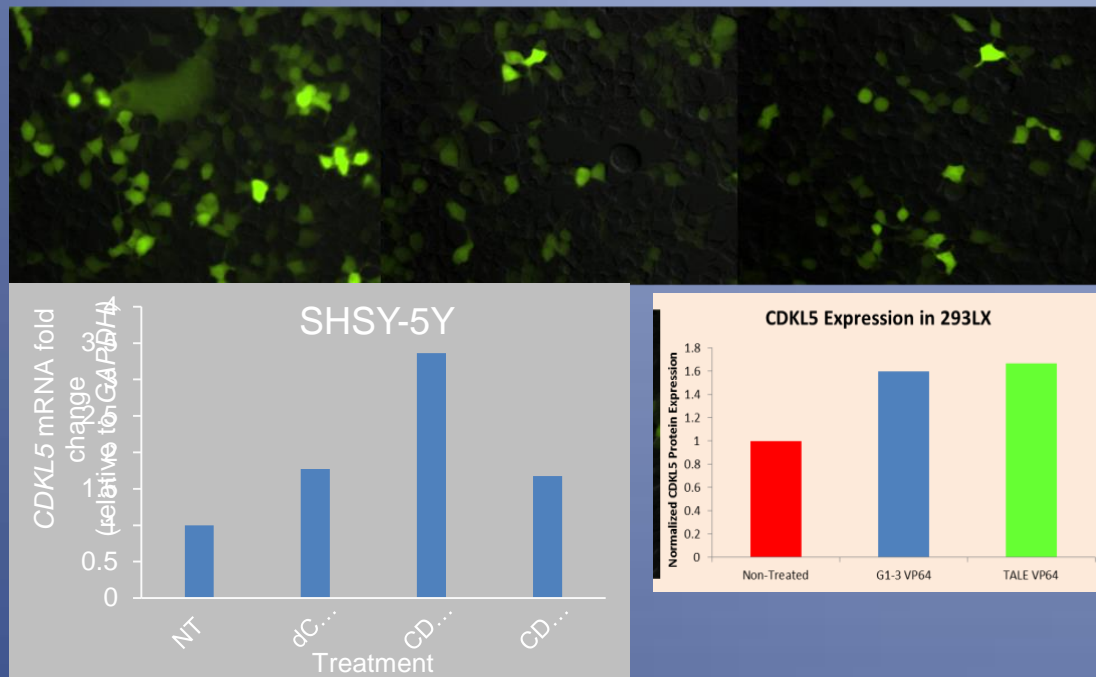


Toxicity screen following injection of LNP encapsulated TALE T3y.

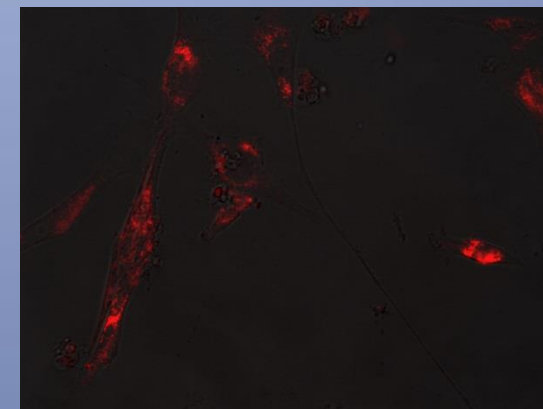


# Other Disease Indications

CDKL5 deficiency  
TALE and dCas9



Angelman Syndrome  
MSC Delivery of S100



Adipose MSC transduced with Lentivirus S100-mCherry



## Gene Activation:

Loss-of-function

- Angelman's Syndrome
  - Zinc Finger approach
- CDKL5 deficiency (infantile epilepsy)
  - TALE and CRISPR activation studies

## Gene Silencing:

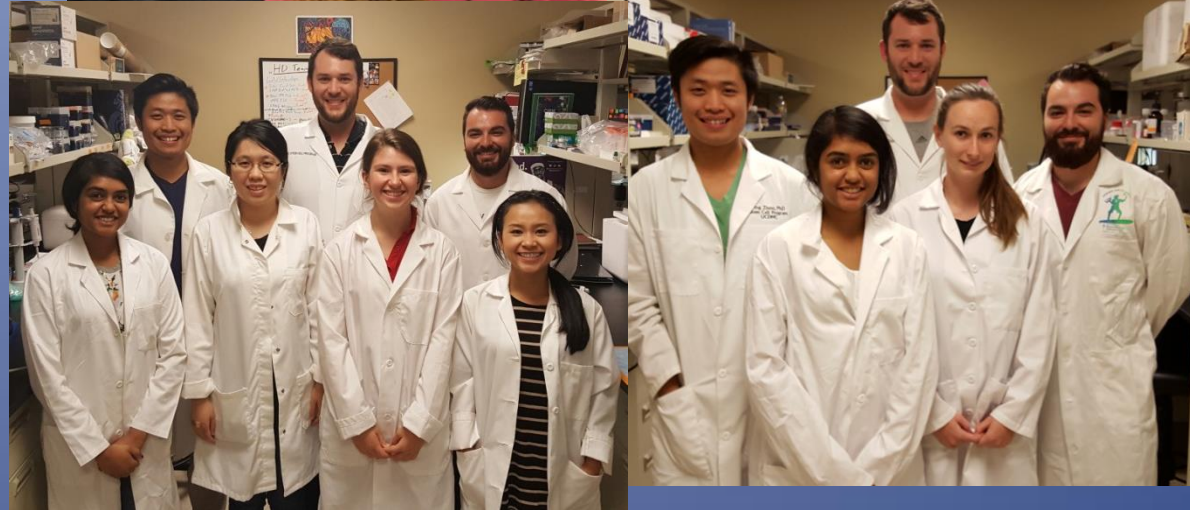
Gain-of-function diseases or disorders

- Huntington's disease
  - TALE and CRISPR silencing studies
- Potential genes implicated in cancer



# Thank you!

- Jan Nolta
  - Director Stem Cell Program
- Vicki Wheelock
  - Director Huntington's disease clinic
- David Segal
  - Associated Director Genome Center
- Peter Deng
- Anvita Komarla
- Joey Aprile
- Megan Cheng
- Sharon Burk
- Thuy Nguyen
- Jasmine Carter
- Sakereh Carter



- *Support for this project was provided by a NIH NRSA Postdoctoral Fellowship F32NS090722 (Fink)*
- *NIH Director's transformative award 1R01GM099688 (Nolta)*
- *NIH NIGMS Predoctoral Fellowship T32GM099608 (Deng)*
- *The Stewart's and Dake Family Gift (Nolta/Fink)*
- *Help4HD International*
- *LouLou Foundation, UPenn Orphan Disease Center (Nolta/Fink)*
- *Philanthropic donors from the HD community, including the Roberson family, WeHaveAFace.org and TeamKJ*