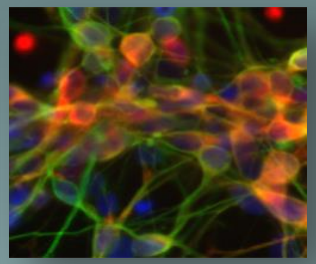
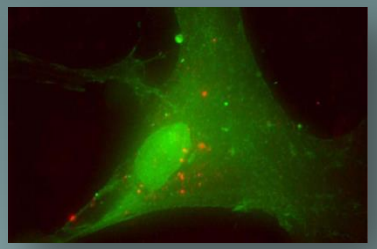
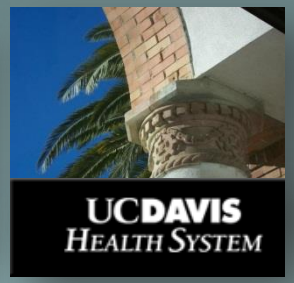


A Novel Therapeutic Approach for HD: Specific Gene Editing Strategies

Northern California HDSA

2016 Chapter Convention

Kyle Fink, PhD

JHD and NeuroGenetics Team

Stem Cell Program

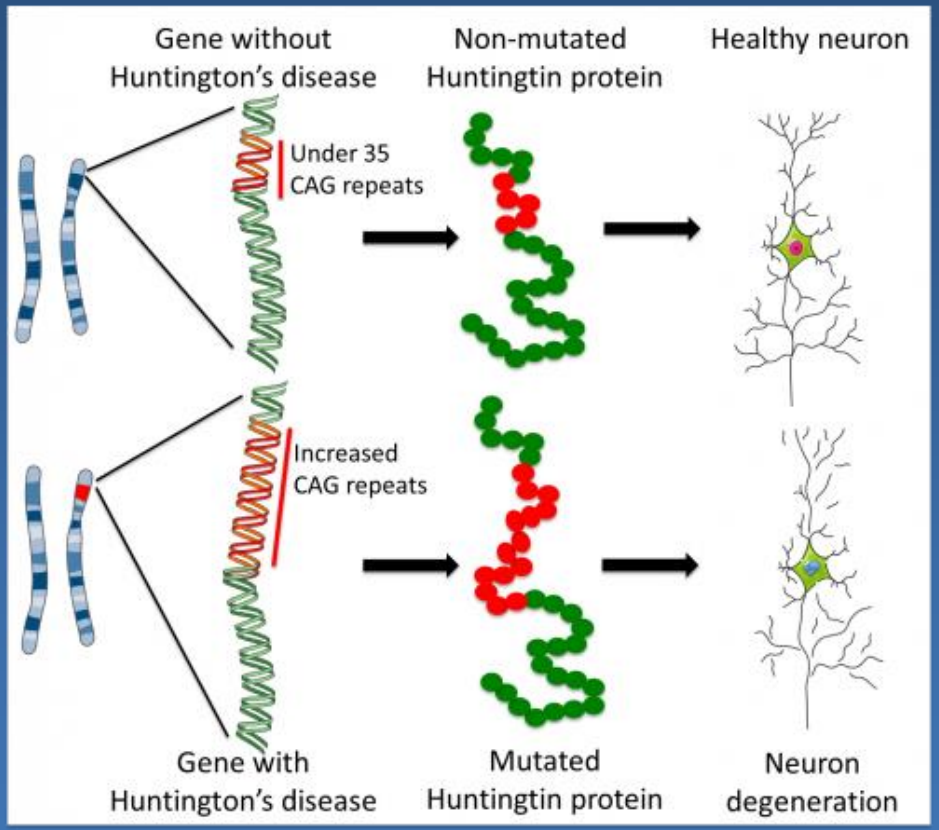
Institute for Regenerative Cures

University of California, Davis Medical Center

Overview

- Experimental Treatment options for Juvenile Huntington's disease
  - Potential Targets for gene therapy
- Transcription Activator-like Effectors
  - Application to JHD
- Preliminary Findings
  - *Publication in Cell Transplantation*
- Future Directions

HTT gene → htt protein



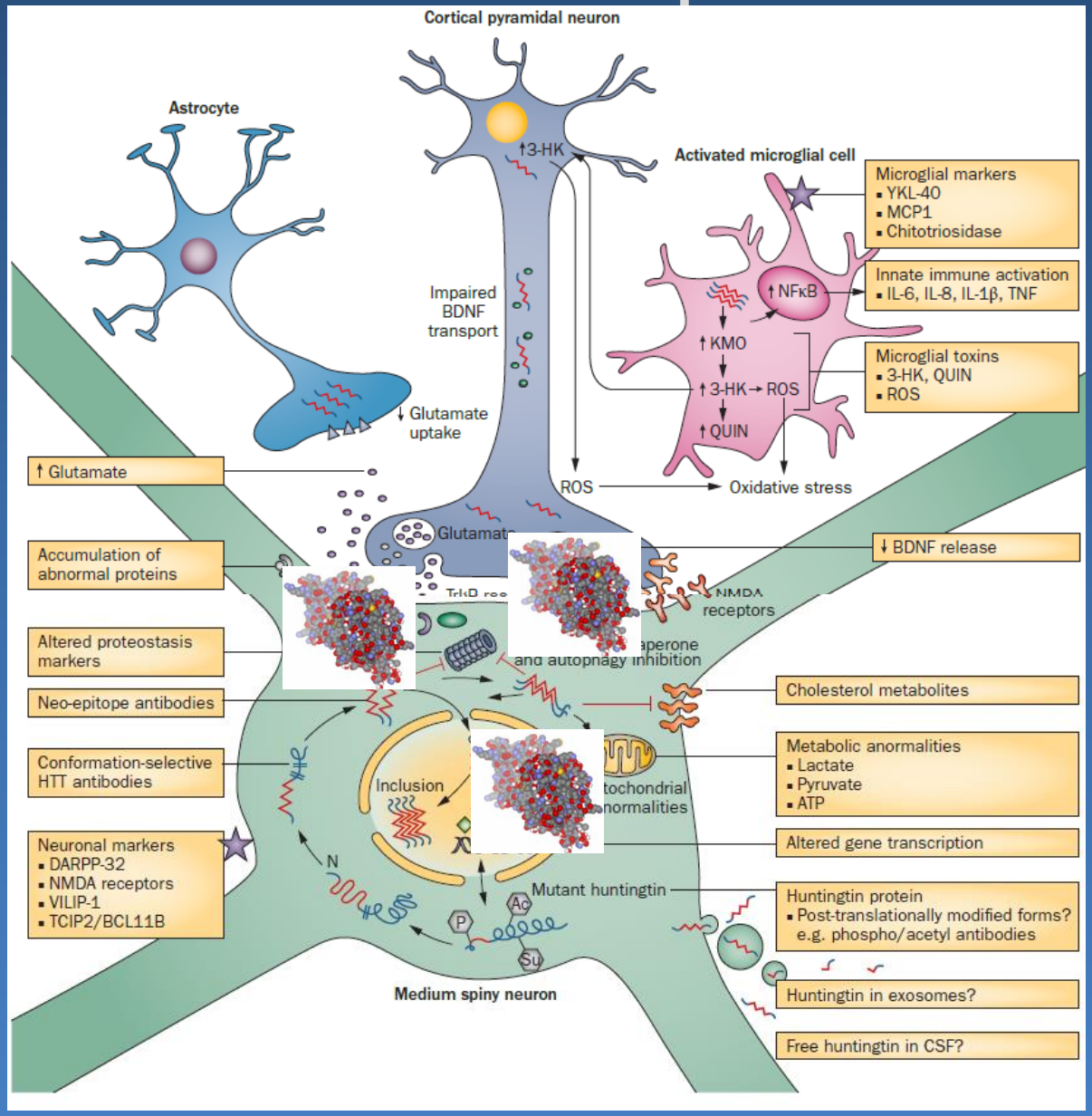
Normal number:	< 31 CAG
"Gray area":	32-38 CAG
Huntington disease:	> 38 CAG

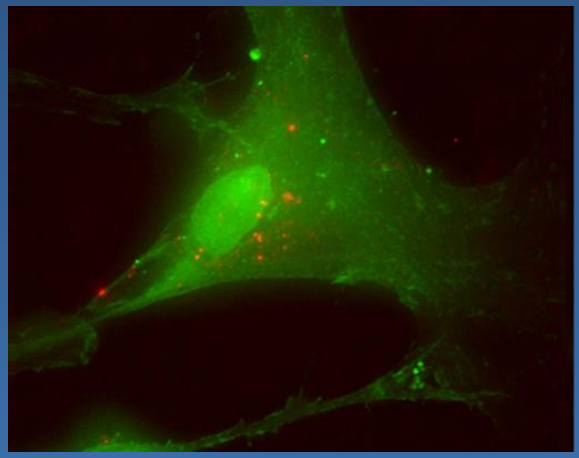
HD Collaborative Research Group. Cell 1993:72; 971–983.

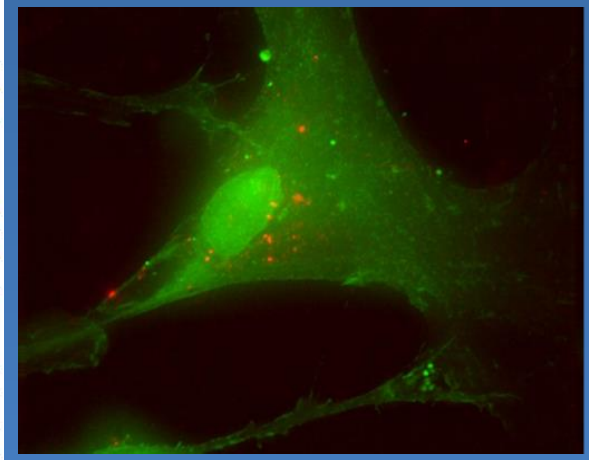
Treatment Options

- Neuroprotection
  - Self regrowth of lost neurons
- Cell Replacement
  - Transplantation of cells that will grow into neurons
- Gene Modification/Correction
  - Silence the mutant gene

Neuroprotection

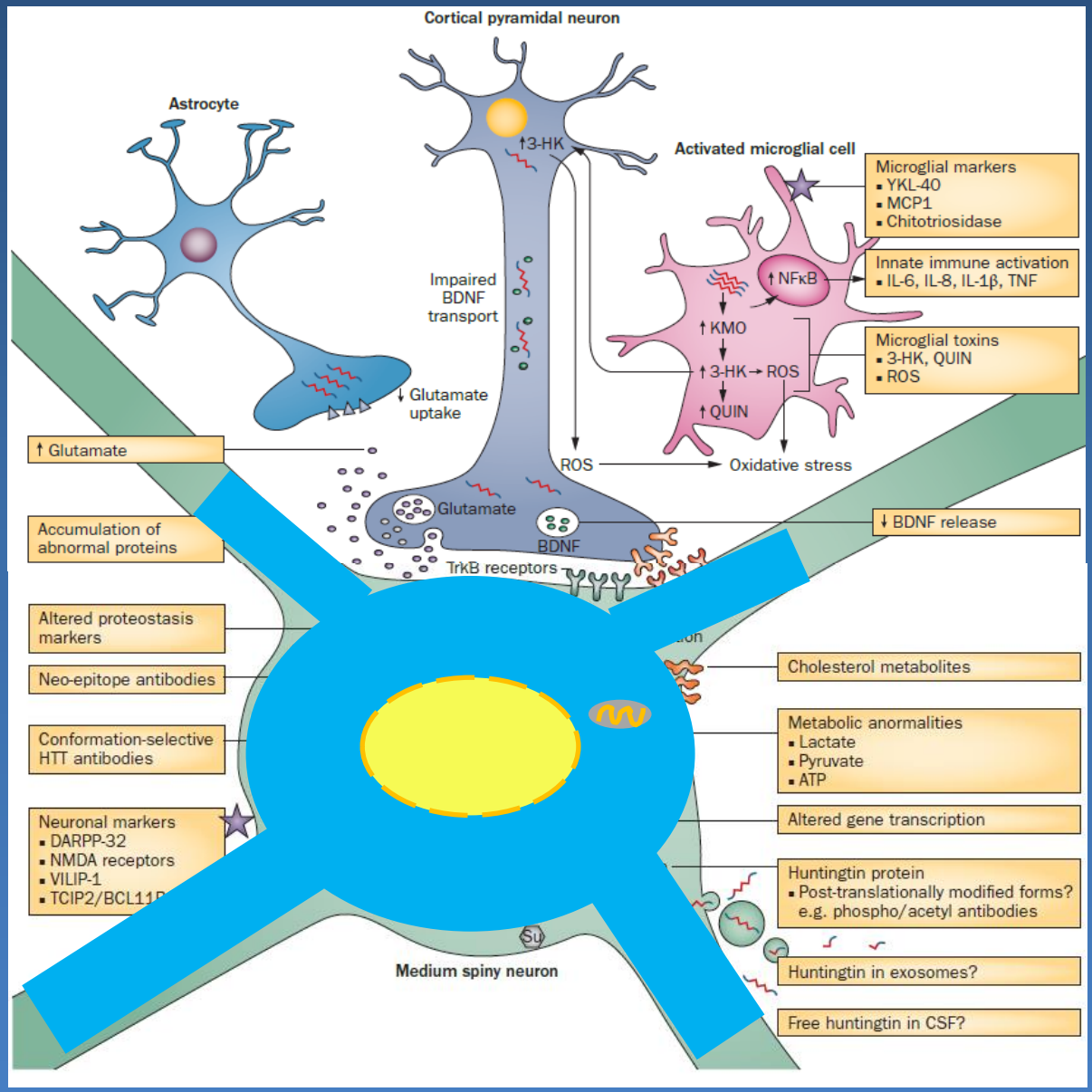






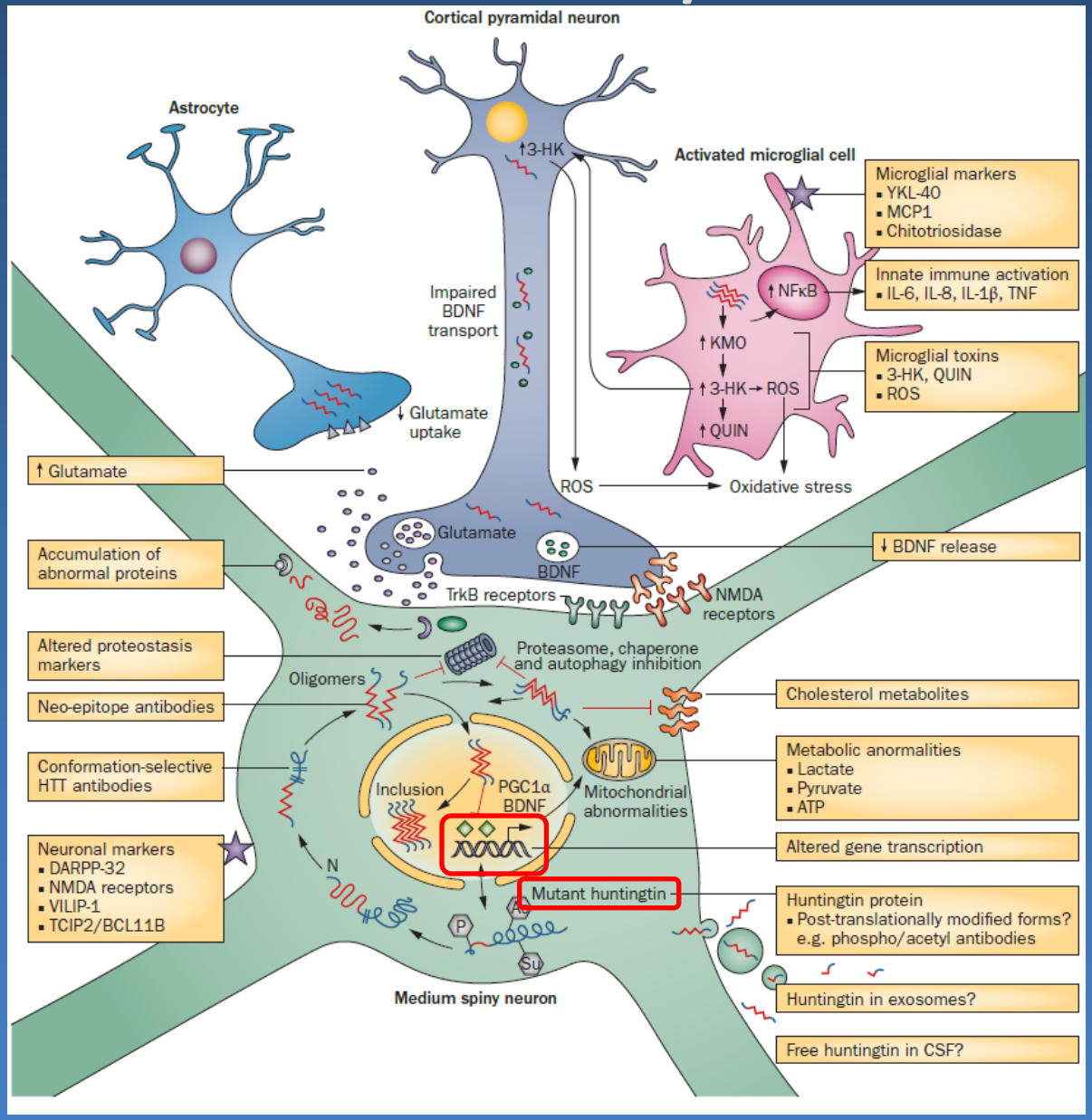
Ross et al, 2014

Cell Replacement



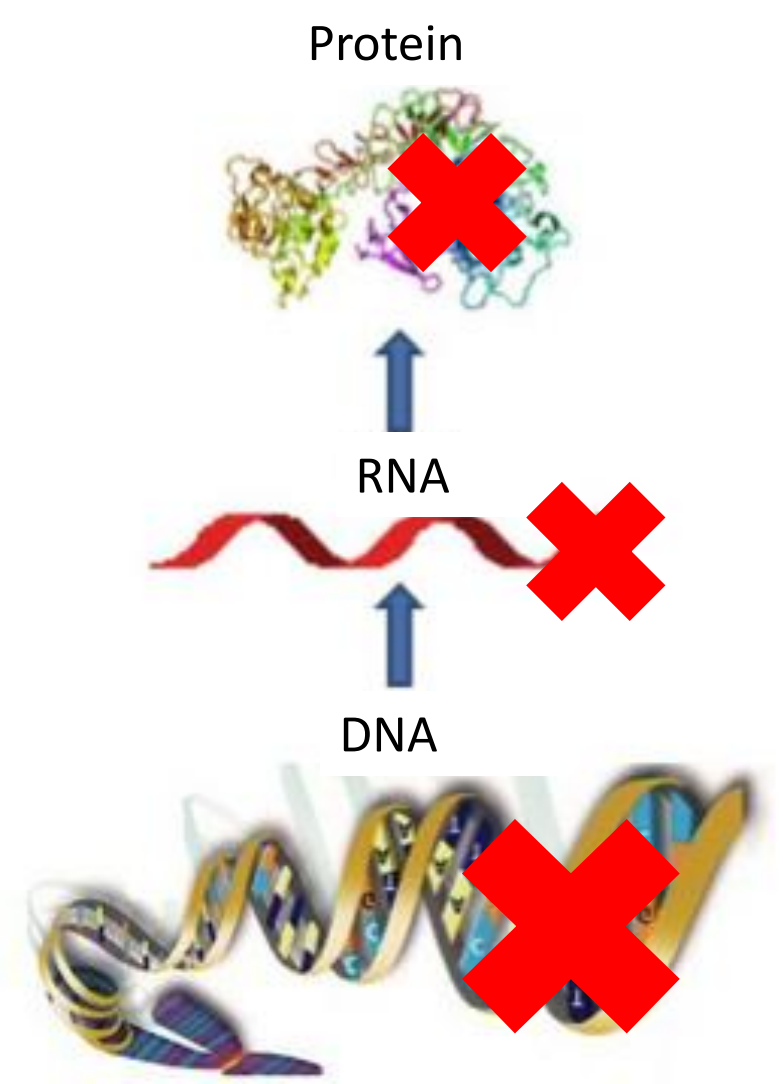
Ross et al, 2014

Gene Modification/Correction



Ross et al, 2014

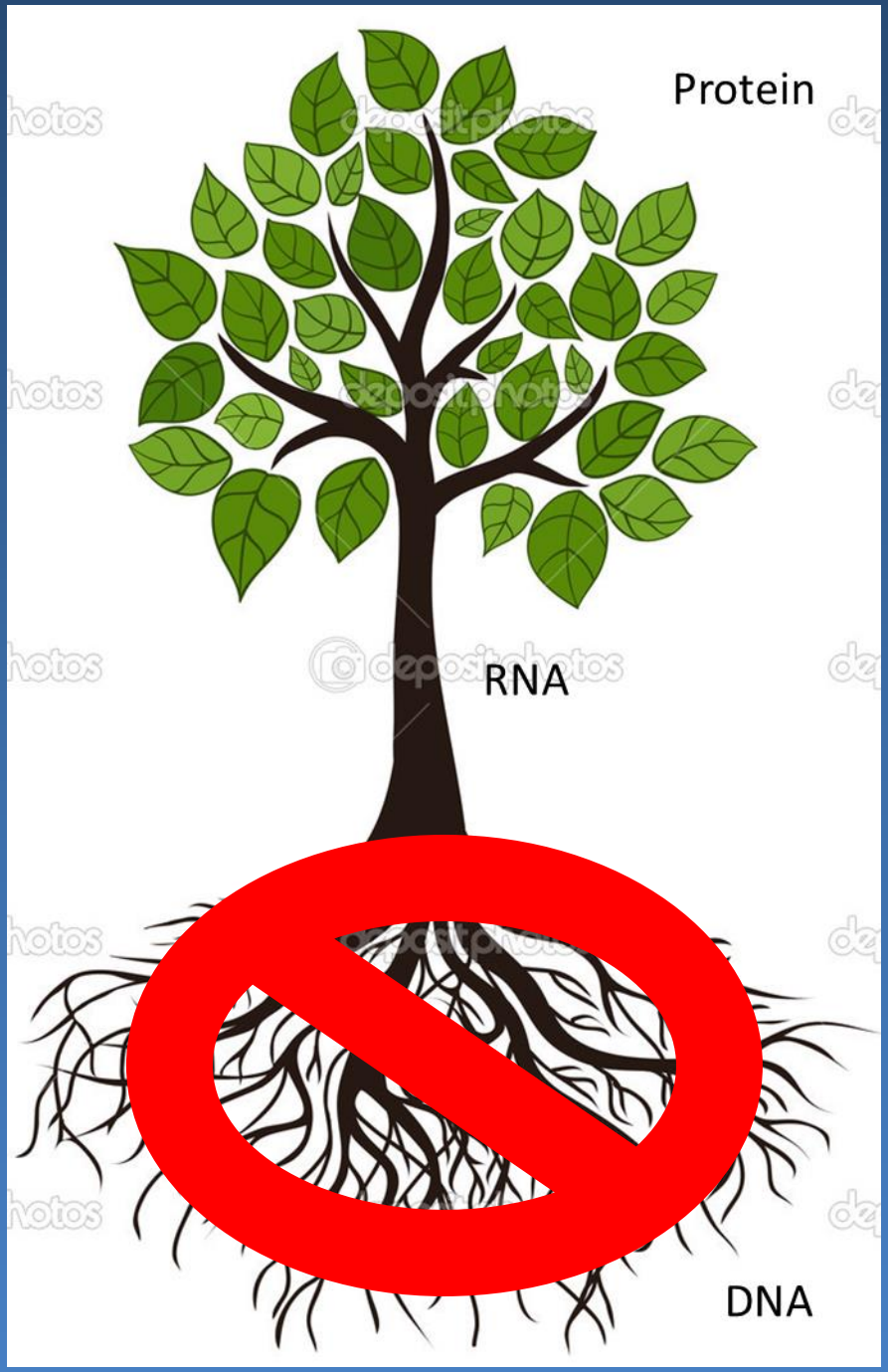
Targets for Reducing mHtt



Degrading the toxic protein and getting rid of it from the cell

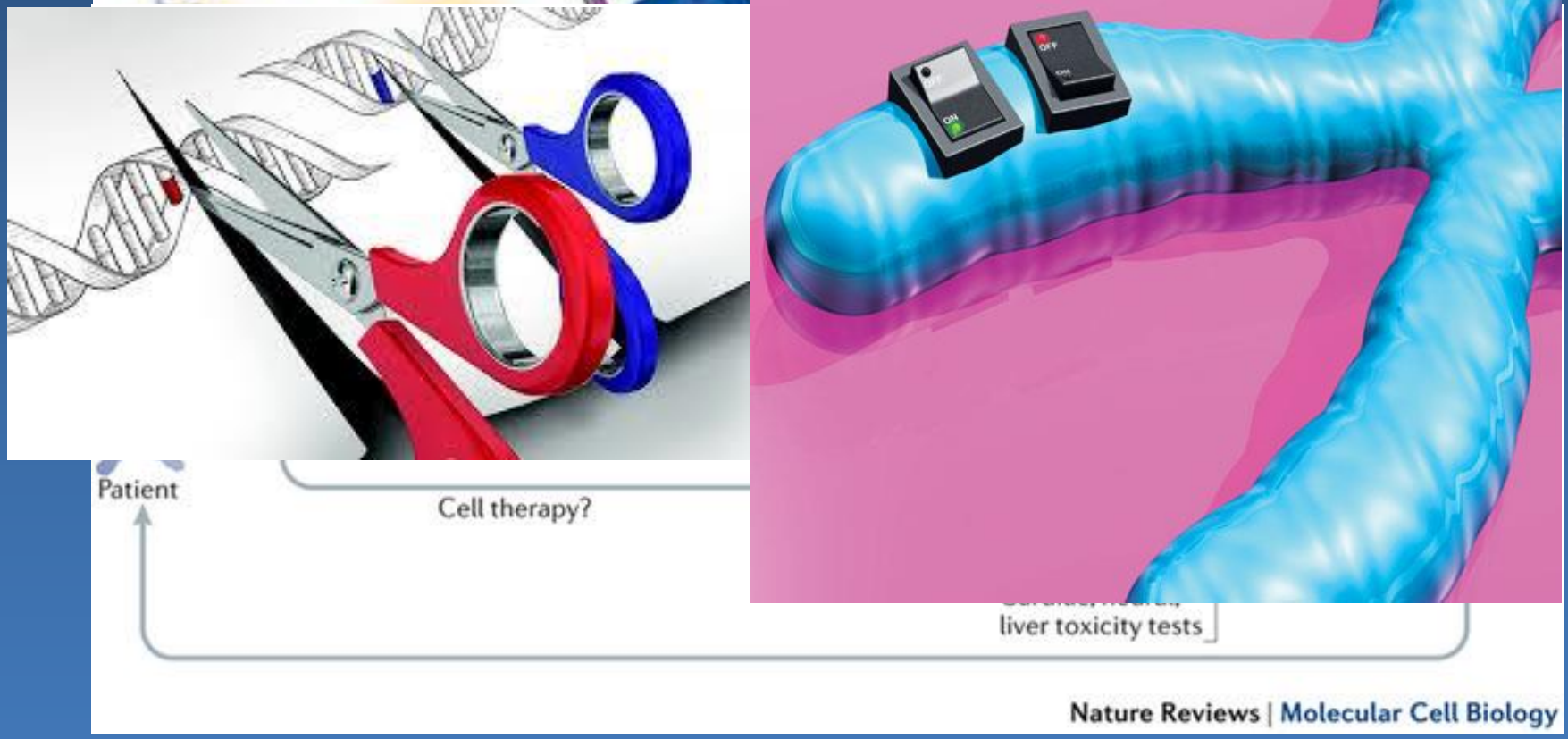
Disrupt mRNA so that it never gets translated into the toxic protein

Silencing the mutant allele to prevent transcription of any mHtt mRNA or protein


Protein
RNA
DNA

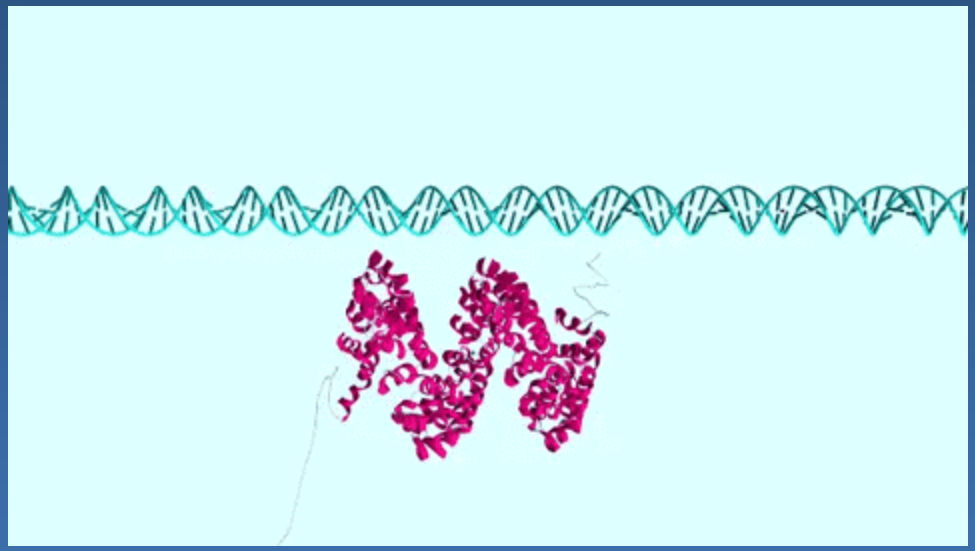
Gene Therapy

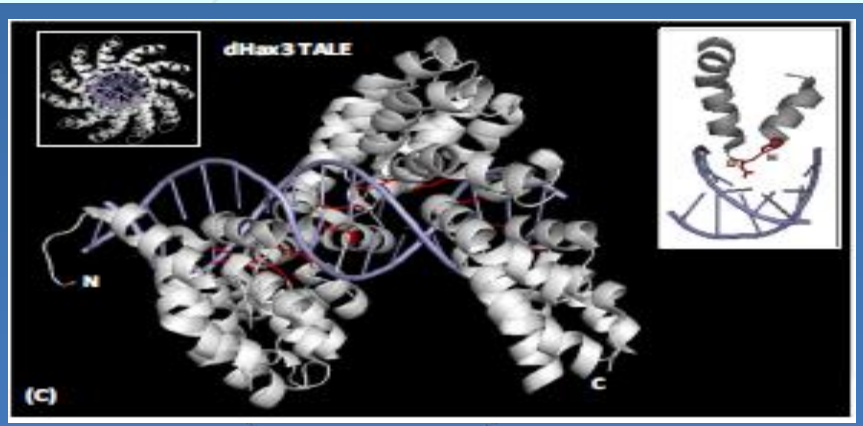
- Traditionally thought of as the addition of a gene that is lacking in a specific disease.
  - Delivered via viral vectors
- Genetically reprogramming cells to a different fate for transplantation
  - Creation of pluripotent cells (iPSC) or induced neurons
- Correction or deletion of a gene
  - New technology Zinc Finger, Transcription Activator-like Effector, CRISPR/Cas9

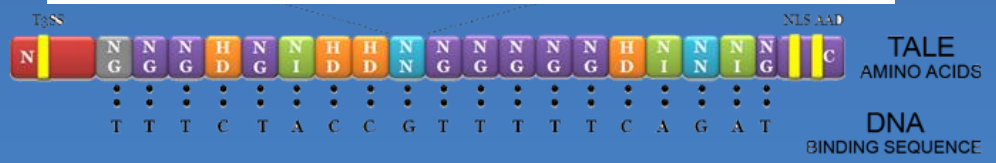

Gene
Therapy
AAV
releases
gene into
cell
Target cell
Receptor
protein
Patient
Cell therapy?
liver toxicity tests
Nature Reviews | Molecular Cell Biology

Transcription activator-like effectors

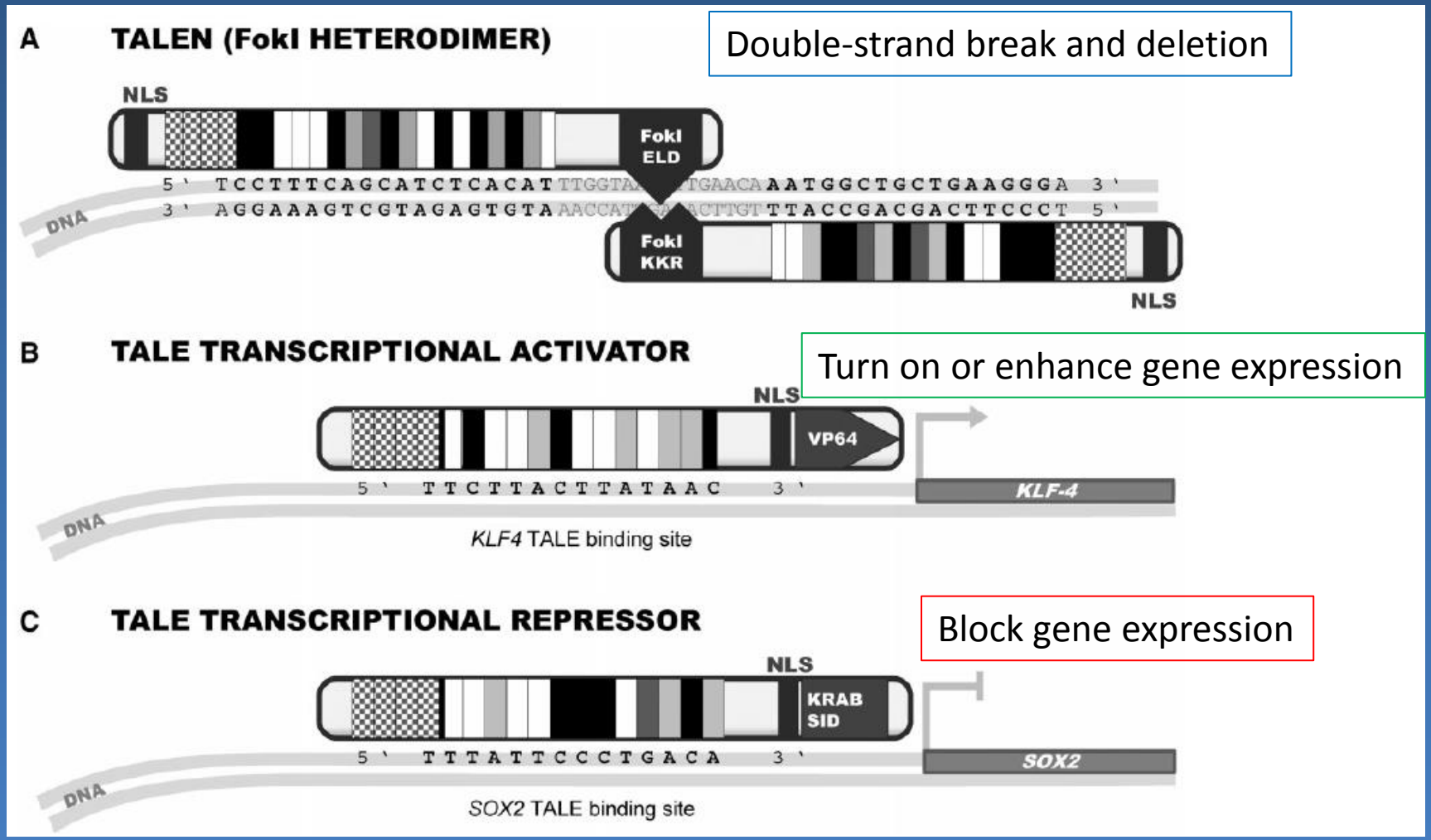
- TALE or TALEN (when paired with a nuclease)
- Derived from plant pathogenic bacteria from the genus Xanthomonas
- One of many DNA-targeting proteins
- Each repeat comprises 33-35 amino acids.
- Can be rapidly synthesized to target any base pair sequence
- Highly efficient and specific with minimal off-target effects
- Can be constructed with a variety of transcription factors (i.e., nucleases, activators, repressors)





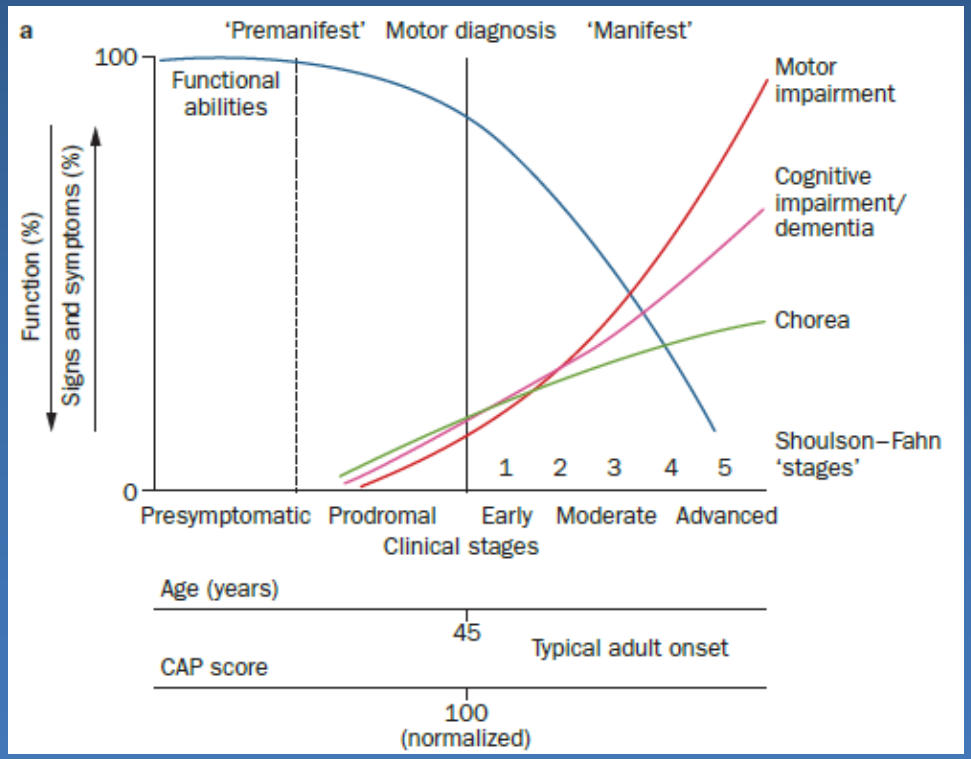


Transcription activator-like effectors

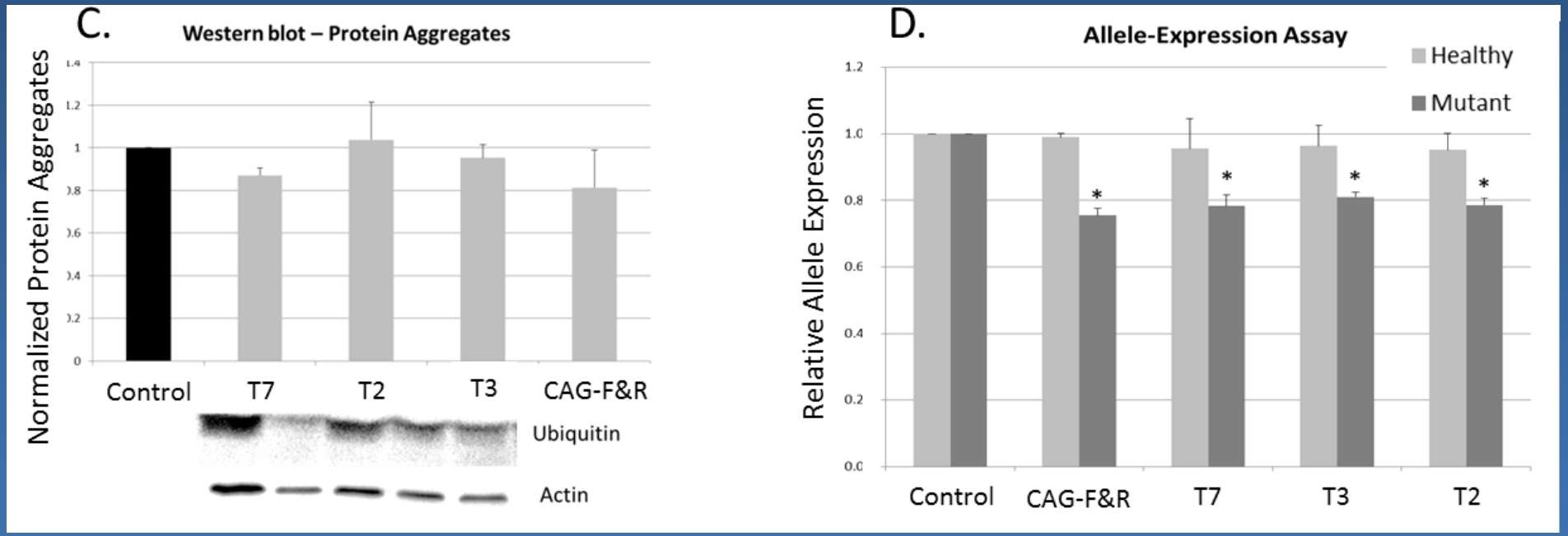


How can we apply this to HD?

- We can Target unique sites in the mutant allele to silence only the mutant allele using gene repression

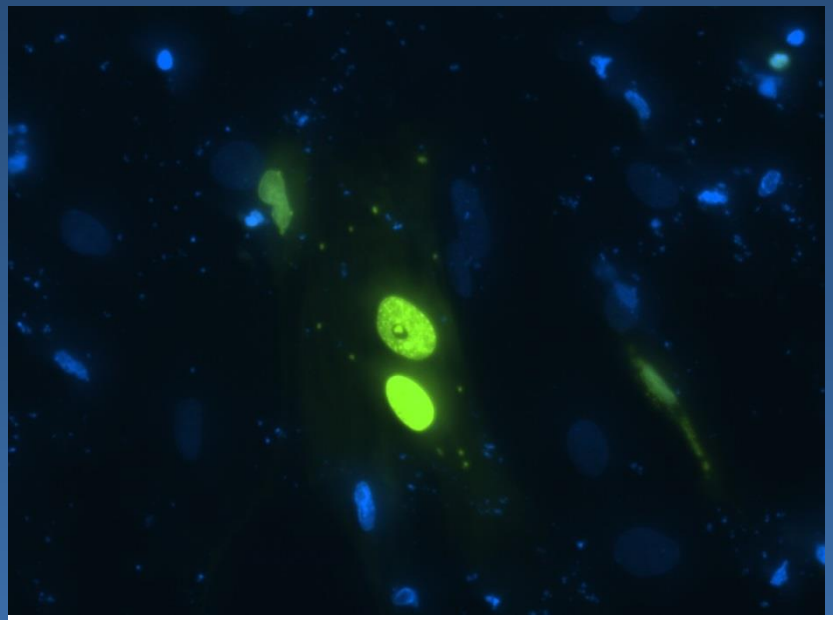


Ross et al, 2014

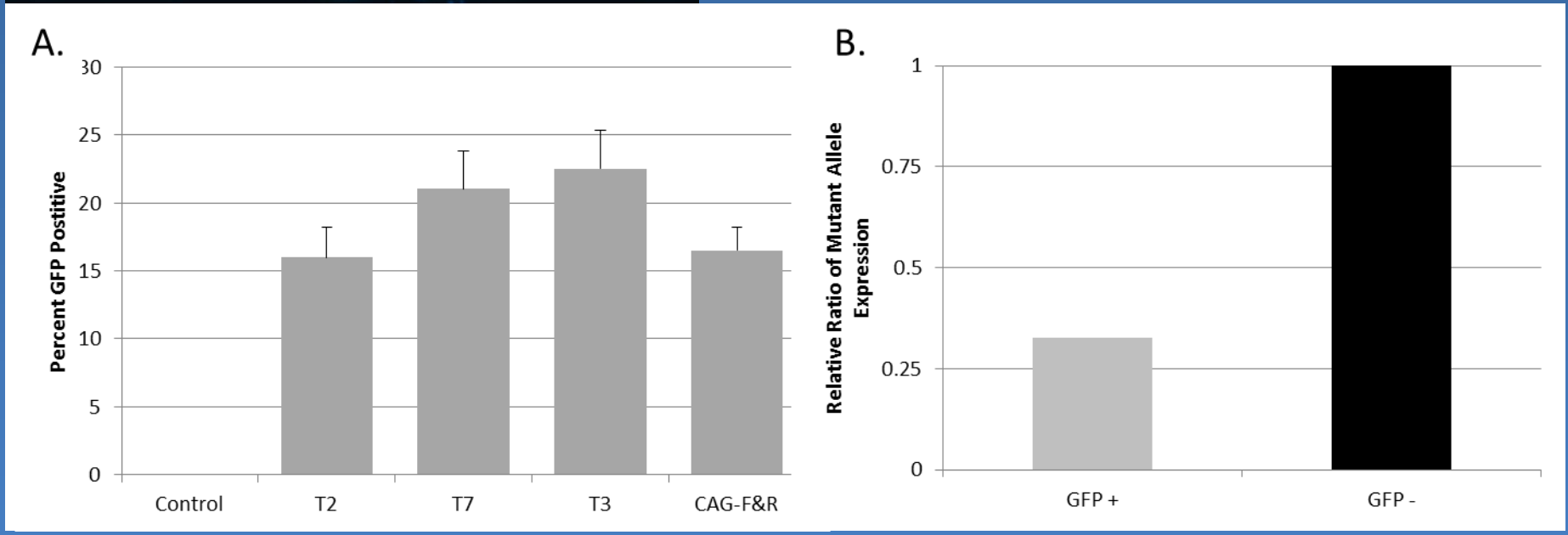


Fink et al, Cell Transplantation, 2016

Transfection Efficiency

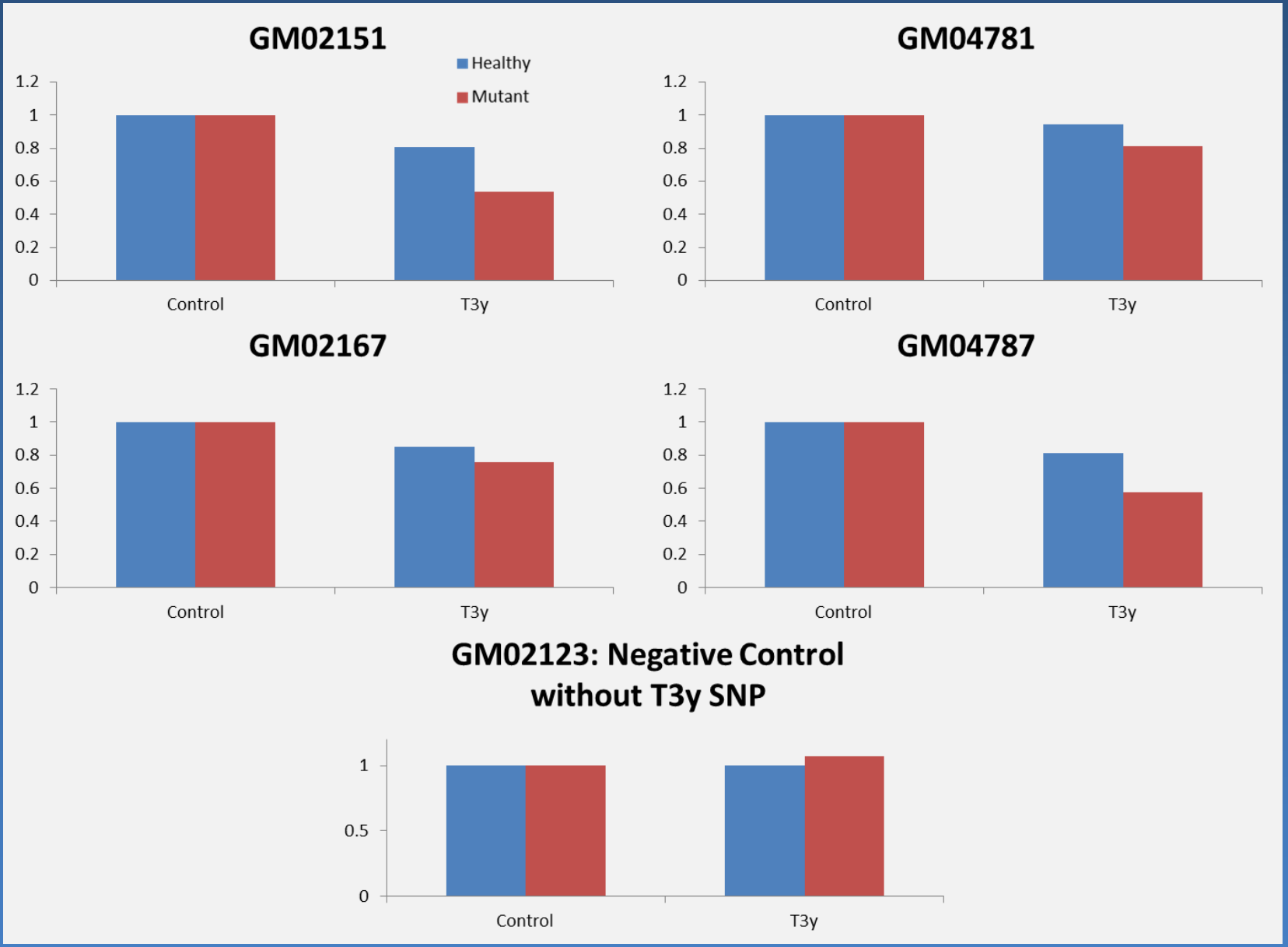


Correlations	GFP/SNP	GFP/Ubi	UBI/SNP
Pgk-Empty	0	0	0
T7	-0.968	-0.652	0.441
T2	-0.855	-0.549	0.035
T3y	-0.951	-0.964	0.999
CAG-F CAG-R	-0.993	-0.999	0.996

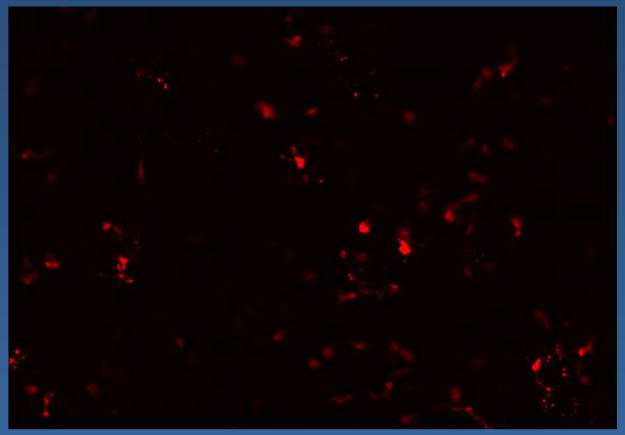


Fink et al, Cell Transplantation, 2016

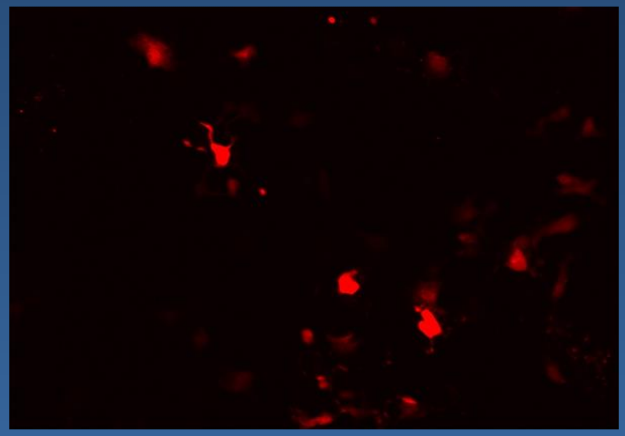
HD Fibro Panel



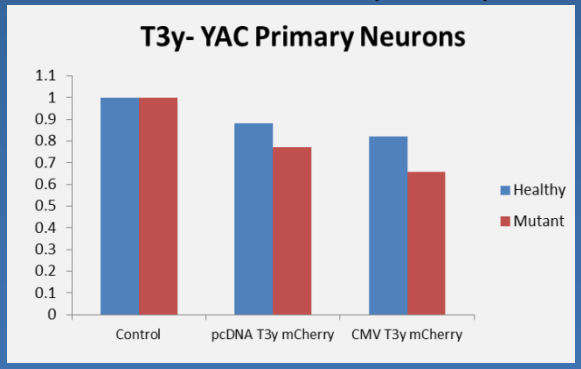
YAC Cultures & Transfection

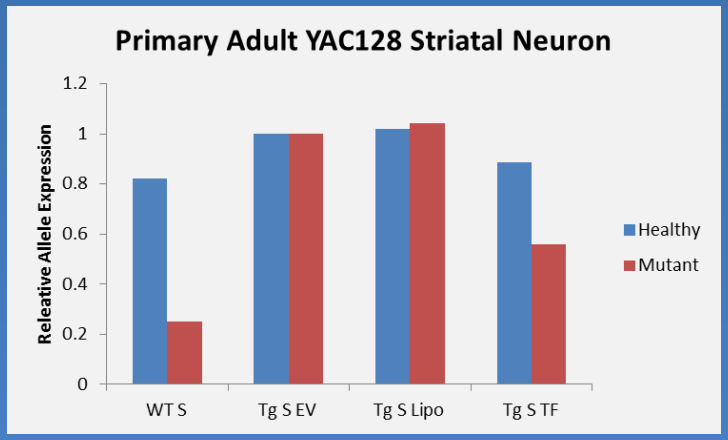


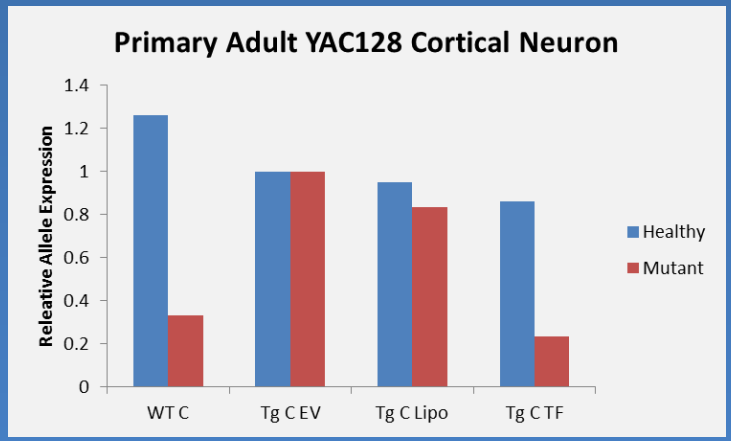
T3y mCherry TreatedYAC128 MSN Culture-10x



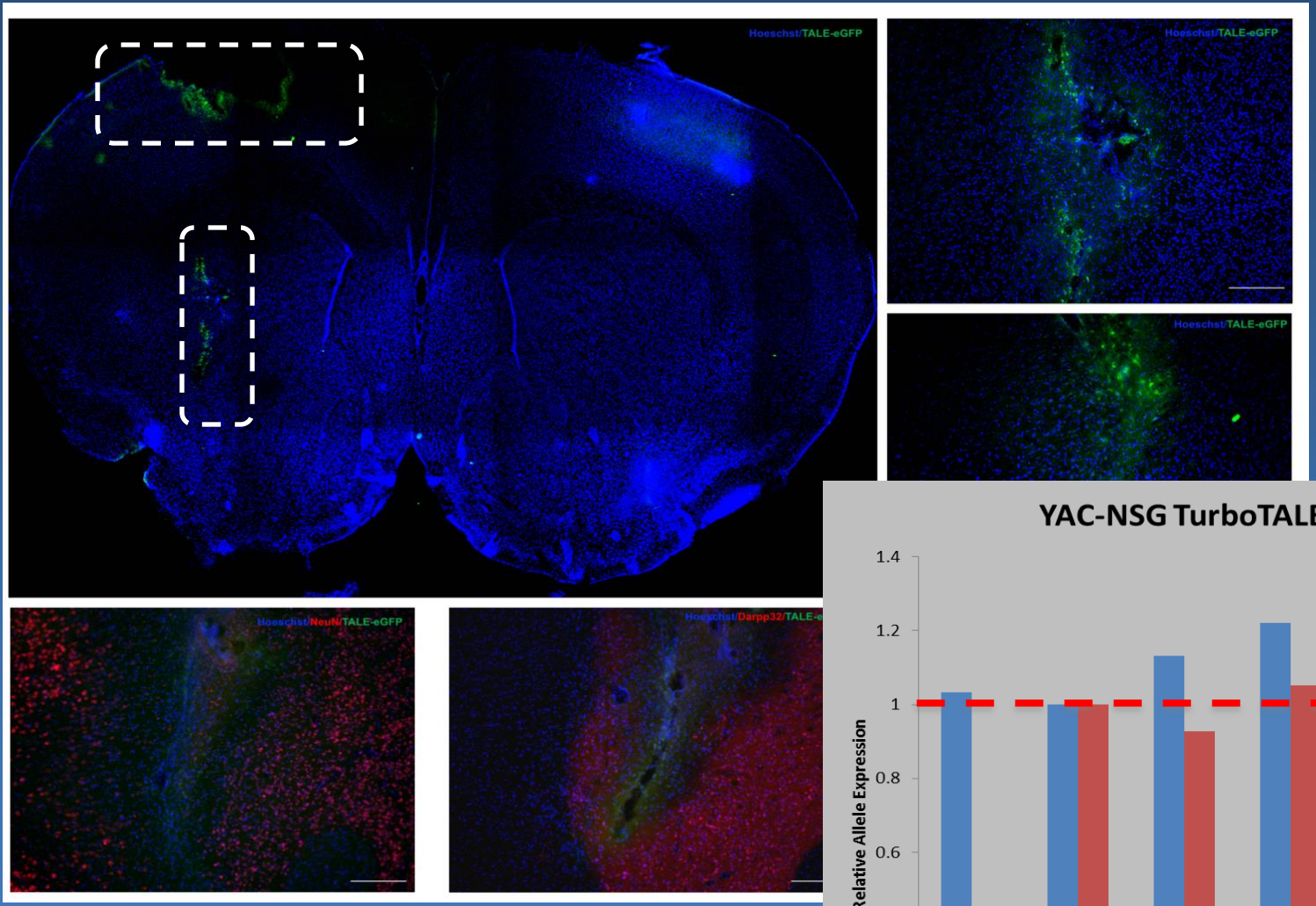
T3y mCherry TreatedYAC128 MSN Culture-20x

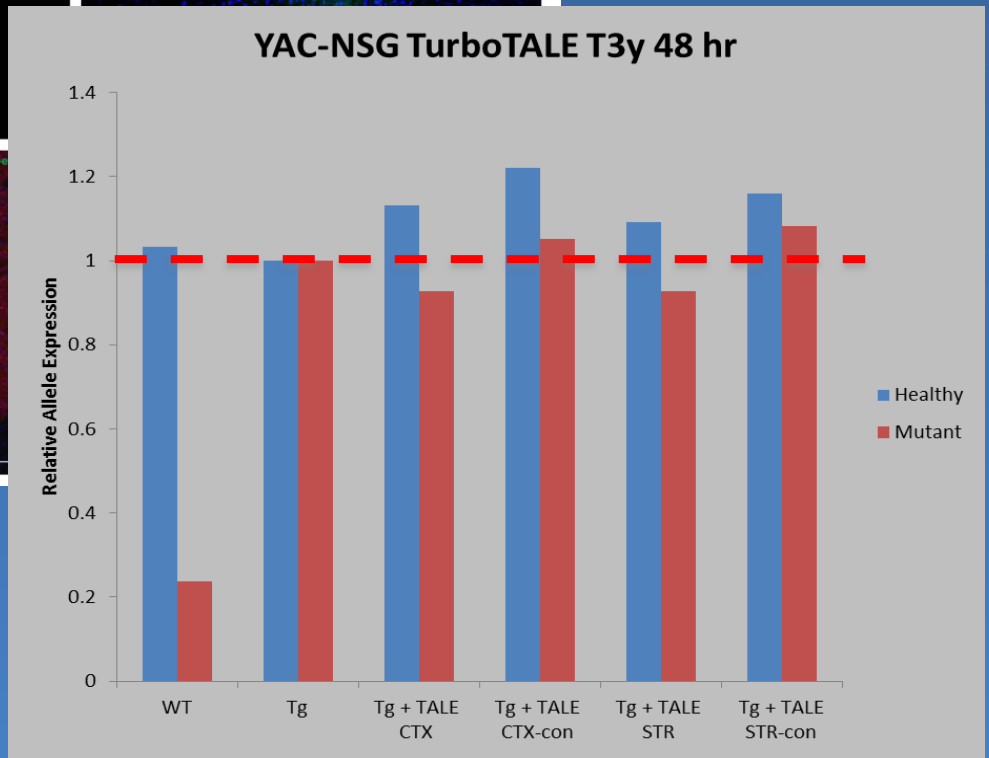






In vivo injection using TurboFect



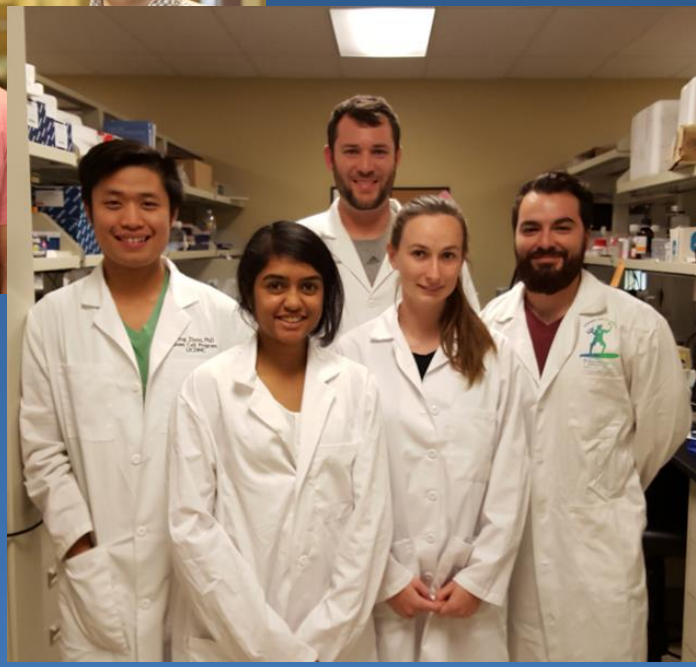


Delivery Options for Gene Therapy

- Delivery of Recombinant Protein
  - Immune response, limited biodistribution
- Delivery via-direct *in vivo* transfection
  - TurboFect (DNA) – limited biodistribution, but observable knockdown
    - Invivofectamine (RNA)– Biodistribution? and possible immune response
- Delivery via viral vectors (AAV) – Fredric Manfredsson MSU Biodistribution in the degenerative brain
  - Immune response with repeated administration?
- Use of synthetic nanoparticles – Precision NanoSystems
  - Unknown biodistribution, immune response and uptake into neurons
- Use of Dendrimer – Julien Rossignol and Ajit Sharma
  - Unknown cellular uptake, biodistribution, and immunolgy
- Use of Mesenchymal Stem Cells as a delivery platform
  - May be able to delivery throughout the brain
  - May create “favorable” microenvironment via immune modulation
  - Able to delivery large proteins to cells of interest
  - Need to “Re-TALE”

Thank you

- Jan Nolta
  - Director Stem Cell Program
- Vicki Wheelock
  - Director Huntington's disease clinic
- David Segal
  - Associated Director Genome Center
- Peter Deng
- Audrey Torrest
- Josh Gutierrez
- Anvita Komarla
- Joey Aprile



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- *California Institute for Regenerative Medicine (CIRM) DR2-05415 (Wheelock/Nolta)*
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- *Philanthropic donors from the HD community, including the Roberson family and TeamKJ*