

# Synthetic biology towards gene therapy: synthetic repressors in Huntington's disease

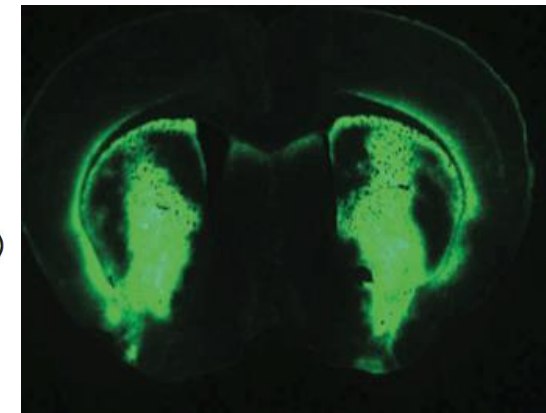
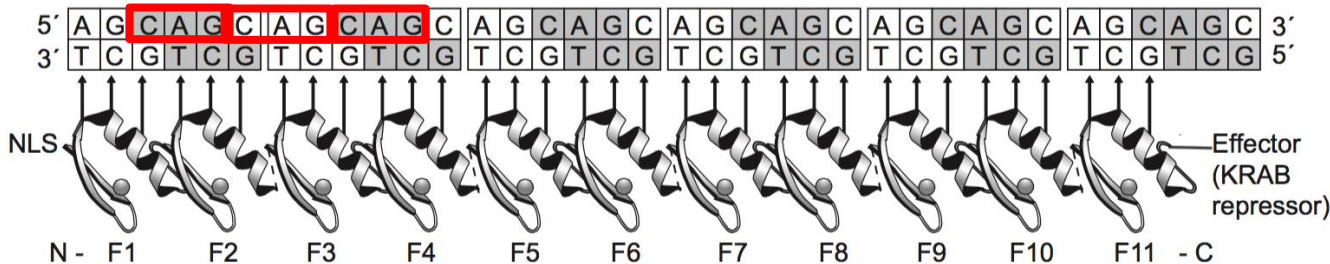
Mark Isalan

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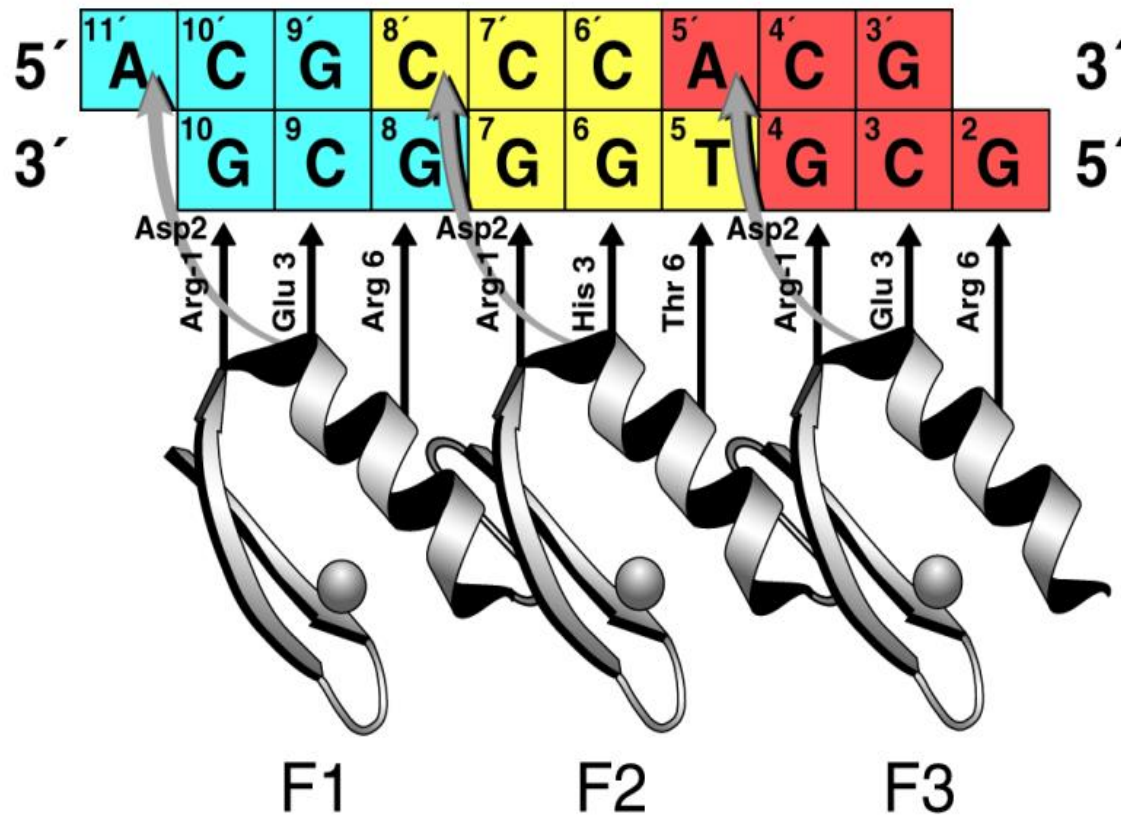
Gene Network Engineering Group  
Imperial College London

wt

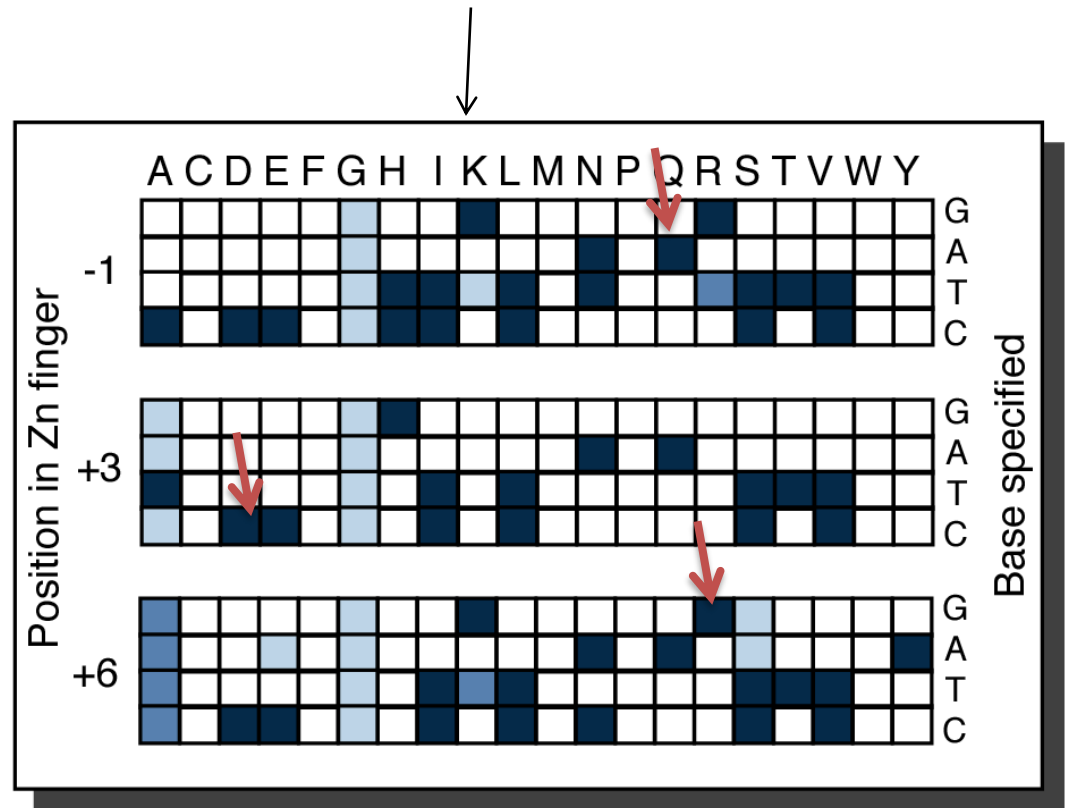
diseased



# Engineering zinc fingers to bind new DNA sequences



# The zinc finger code



e.g. R:g, Q:a, T:t, D:c

**5'-GCA-3'**

# Huntington's Disease

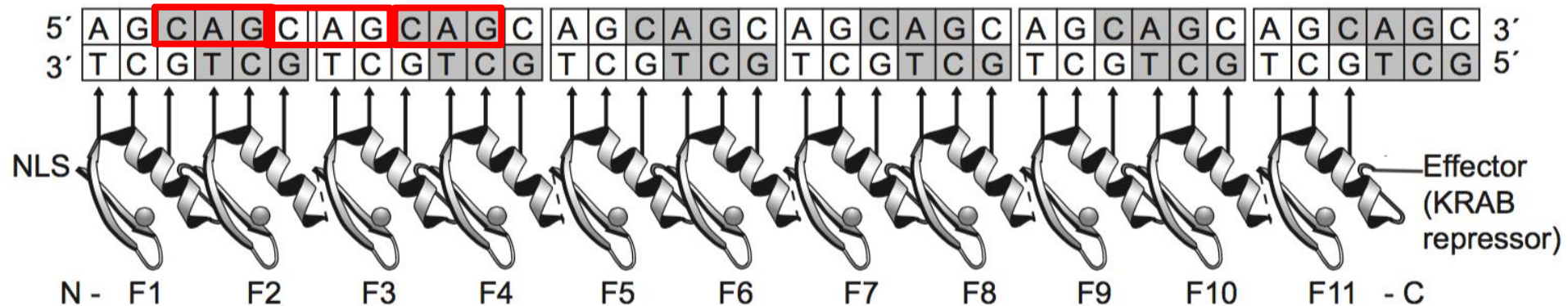
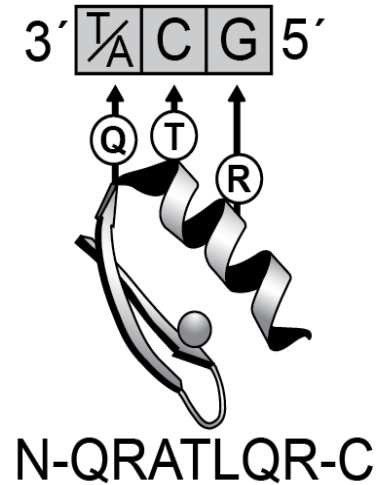
- >1 in 10,000 people (up to 1 in 400 in elderly)
- Autosomal dominant
- Typical onset: 35 to 45 yrs
- Abnormal movements, loss of cognitive function, dementia and death
- Pathology: specific neuronal cell death in striatum and frontal cortex
- Only palliative treatments, although RNAi and antisense are promising



# Zinc fingers to bind poly-CAG

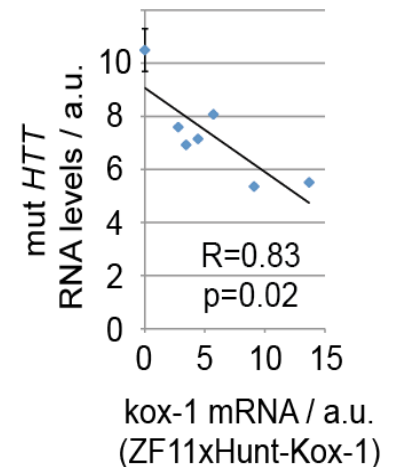
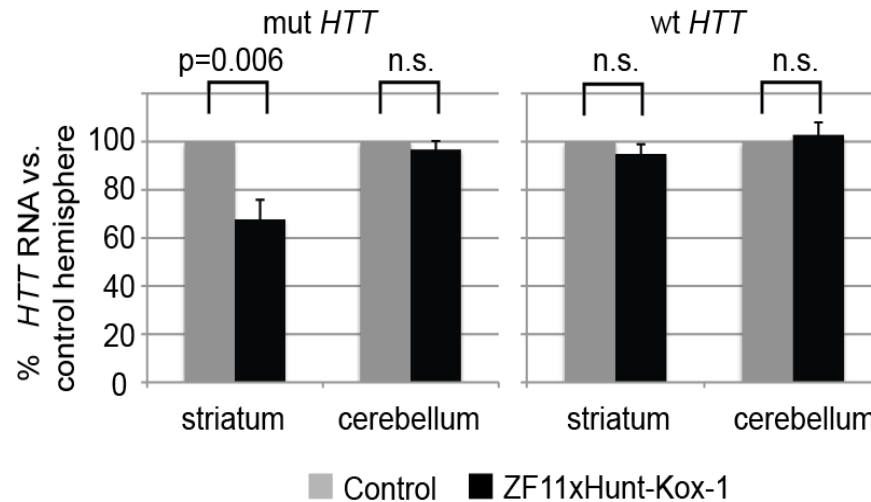
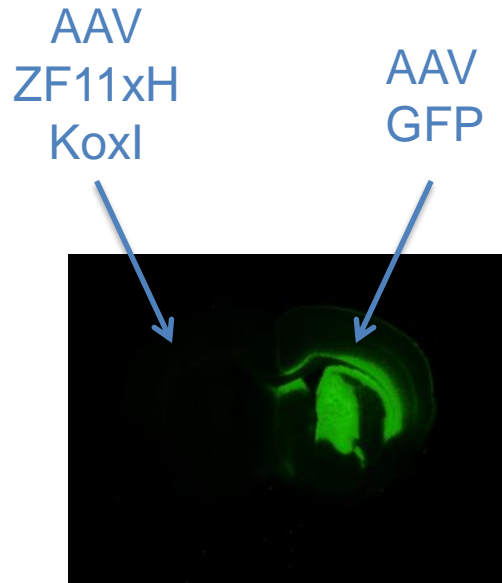
PNAS 109:E3136 (2012)

- Huntington's disease: expanded poly-CAG repeats
- Zinc fingers to bind GCA, GCT (ie CAG)



# ZF11xHunt-Kox1 reduces mut *HTT* mRNA in a dose-dependent manner

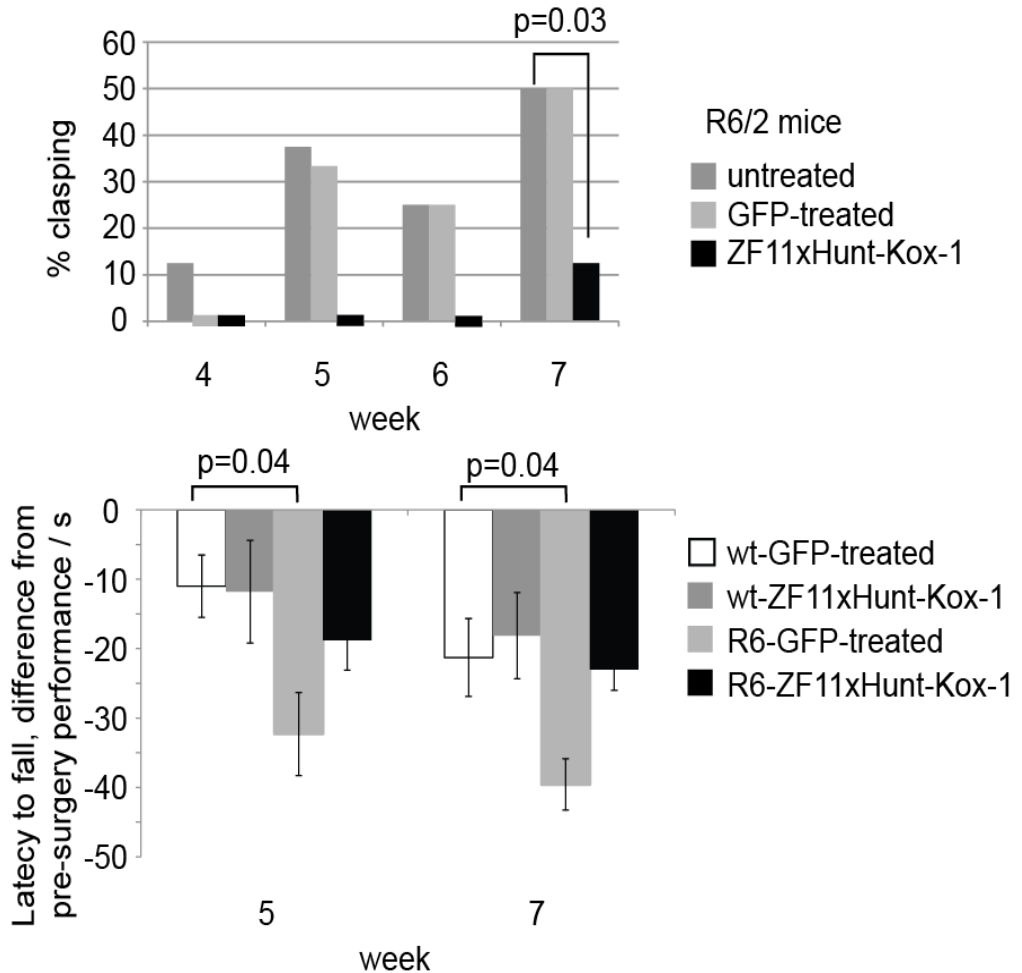
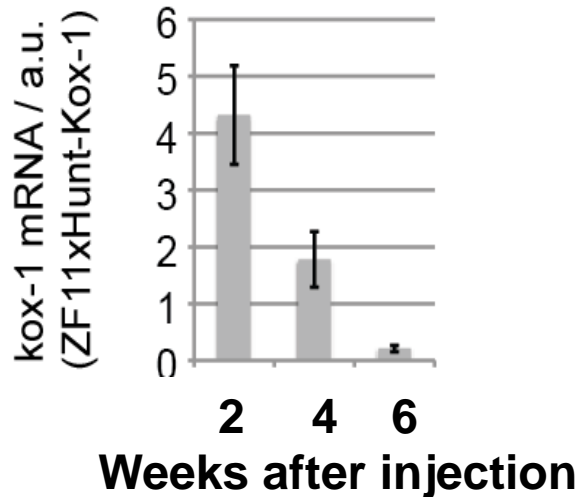
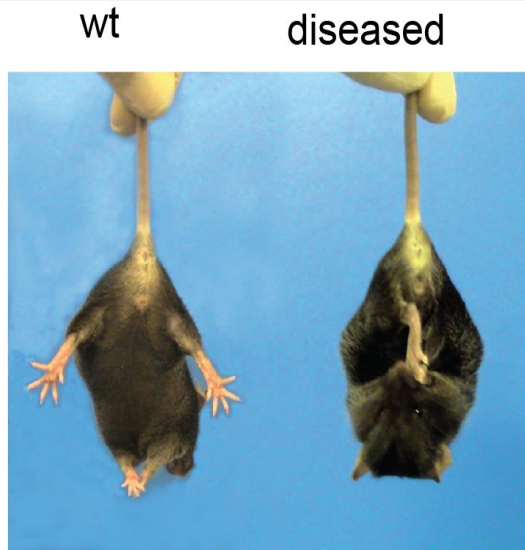
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Treatment  
with AAV2/1  
ZF11-Kox-1  
(2 weeks)



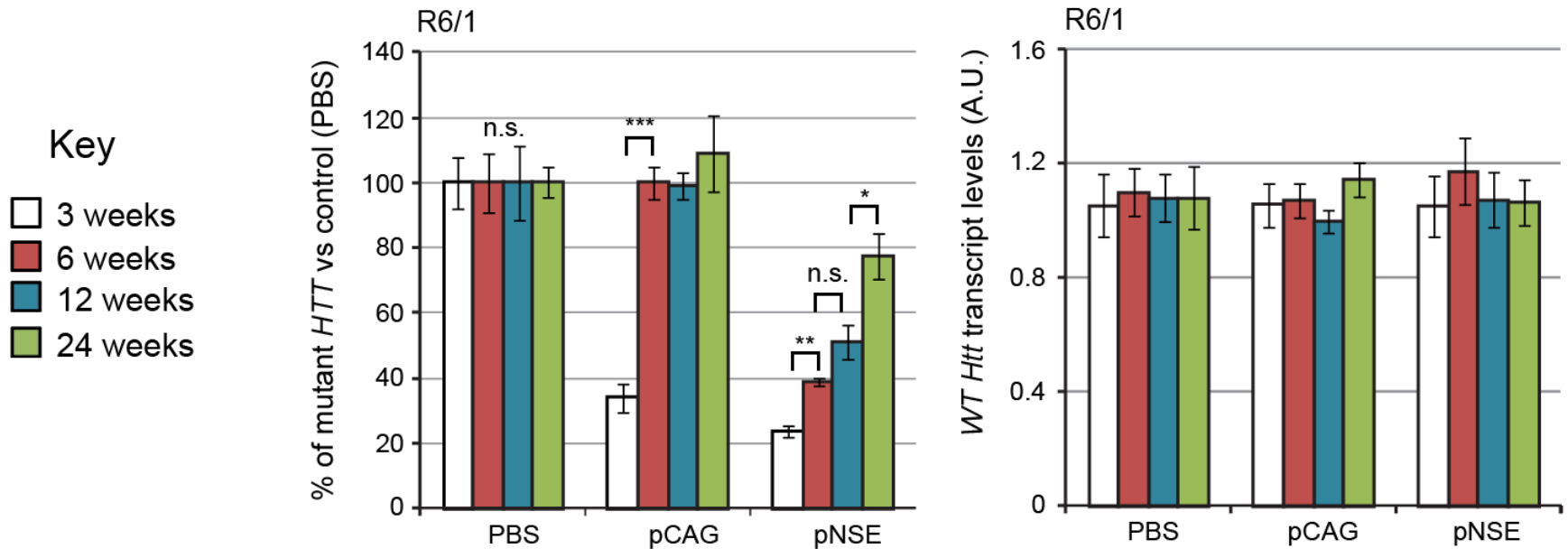
# R6/2 phenotype: clasping and rotarod



**ZF expression transient at this stage**

# pNSE-mZF-KRAB constructs mediate long-term repression in whole brain samples after single intraventricular injections.

Agustín-Pavón C et al. *Molecular Neurodegeneration* 11(1):64 (2016).



Repression of mutant *Htt* ~25% in whole brain after 24 weeks!  
Other genes (incl. mouse endogenous WT *Htt*) unaffected



# Thanks

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Alicia  
Broto

Michal  
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wellcome trust

