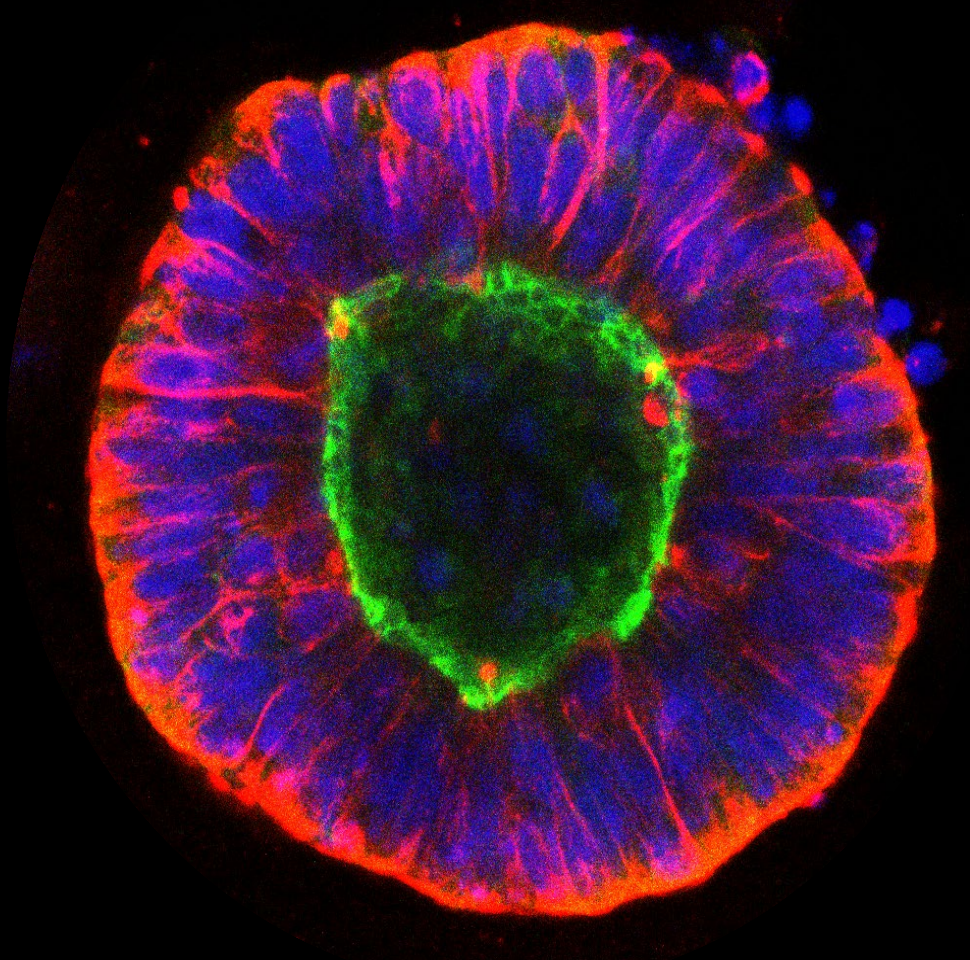
Wild-type Huntingtin, a billion-year experiment



Elena Cattaneo
University of Milano and National Institute of Molecular Genetics

Cell, Vol. 72, 971–983, March 26, 1993, Copyright © 1993 by Cell Press

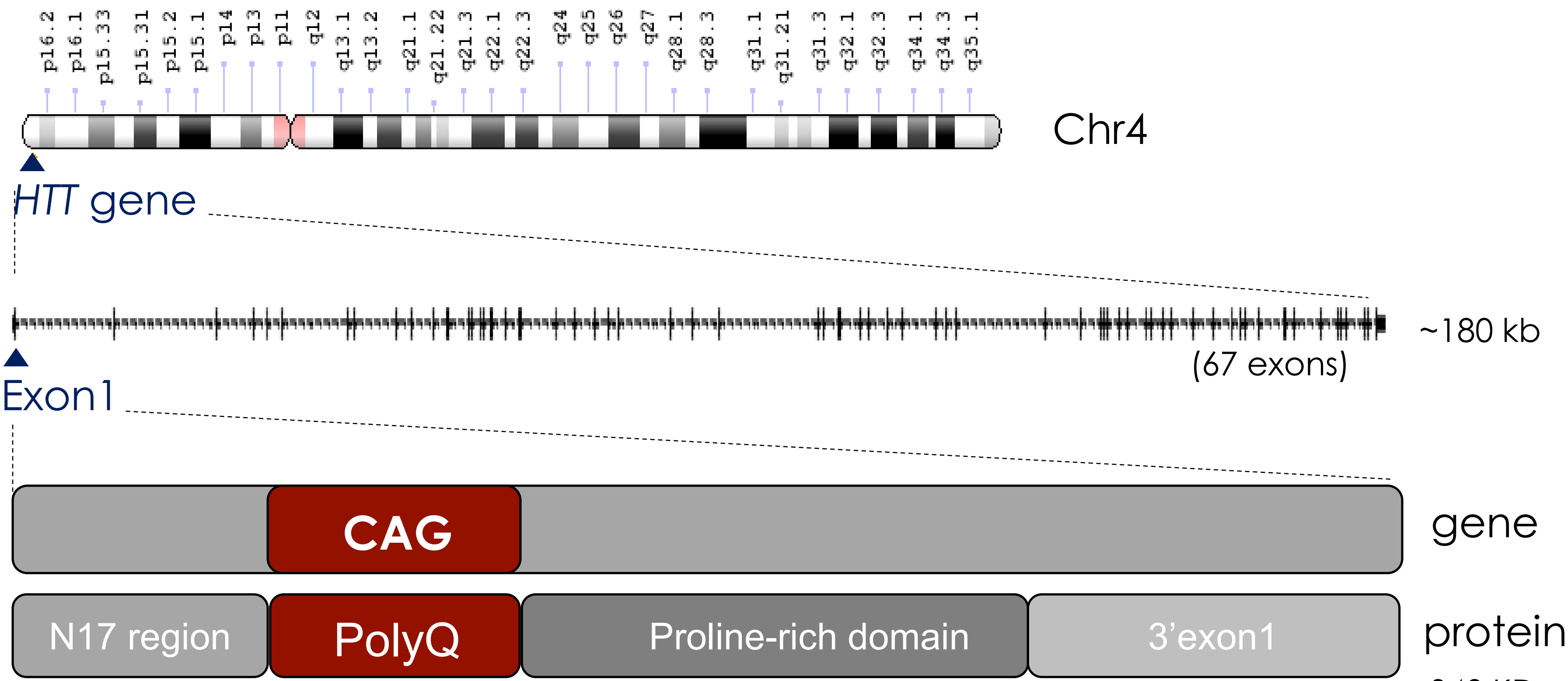
A Novel Gene Containing a Trinucleotide Repeat That Is Expanded and Unstable on Huntington's Disease Chromosomes

The Huntington's Disease Collaborative Research Group*

1979

1983

1993



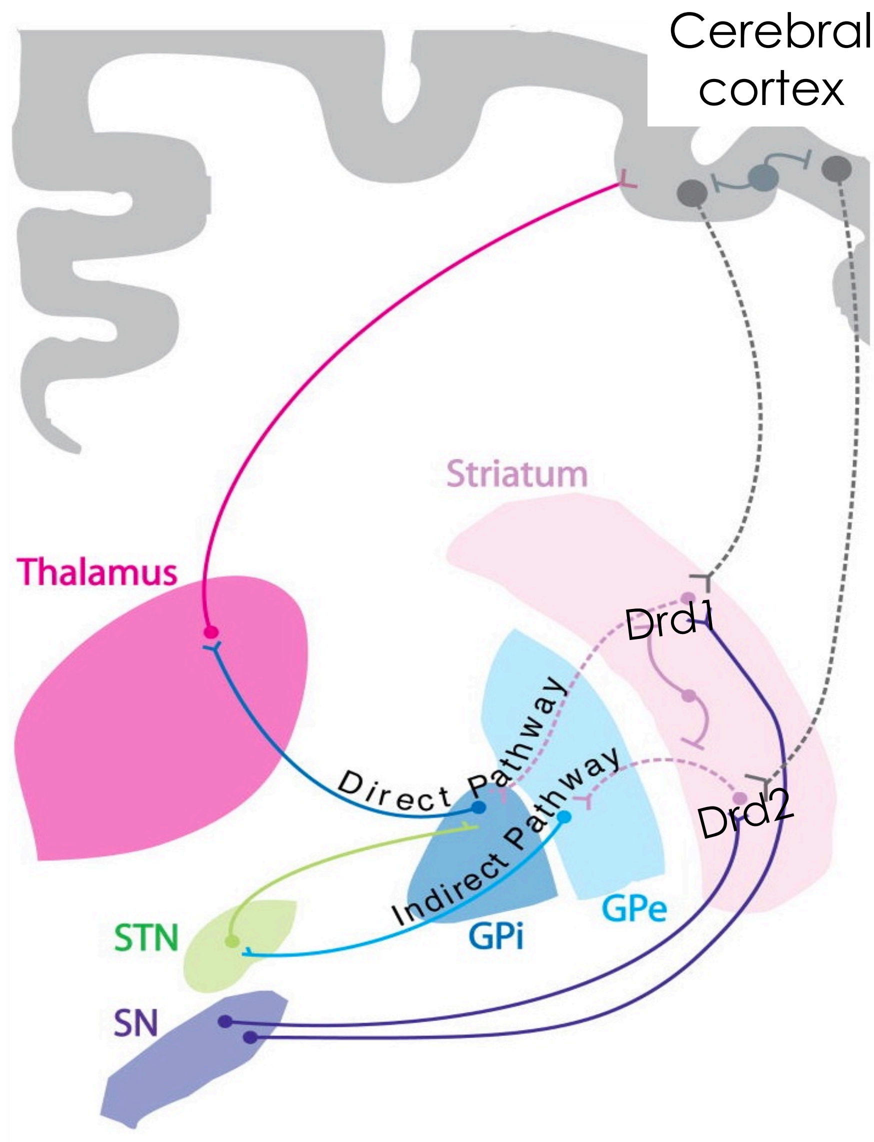
8-35	Q normal repeats
36-39	**Q reduced penetrance**
40-55	**Q repeats adult onset**
60-180	**Q repeats juvenile onset**

Huntington's Disease

- Autosomal dominant, neurodegenerative disease
- Movement disorder, cognitive impairment, mood disturbances
- Midlife onset (juvenile and adult)
- Mortality 15-20 years following onset
- Degeneration of striatal medium spiny
- Cortical atrophy

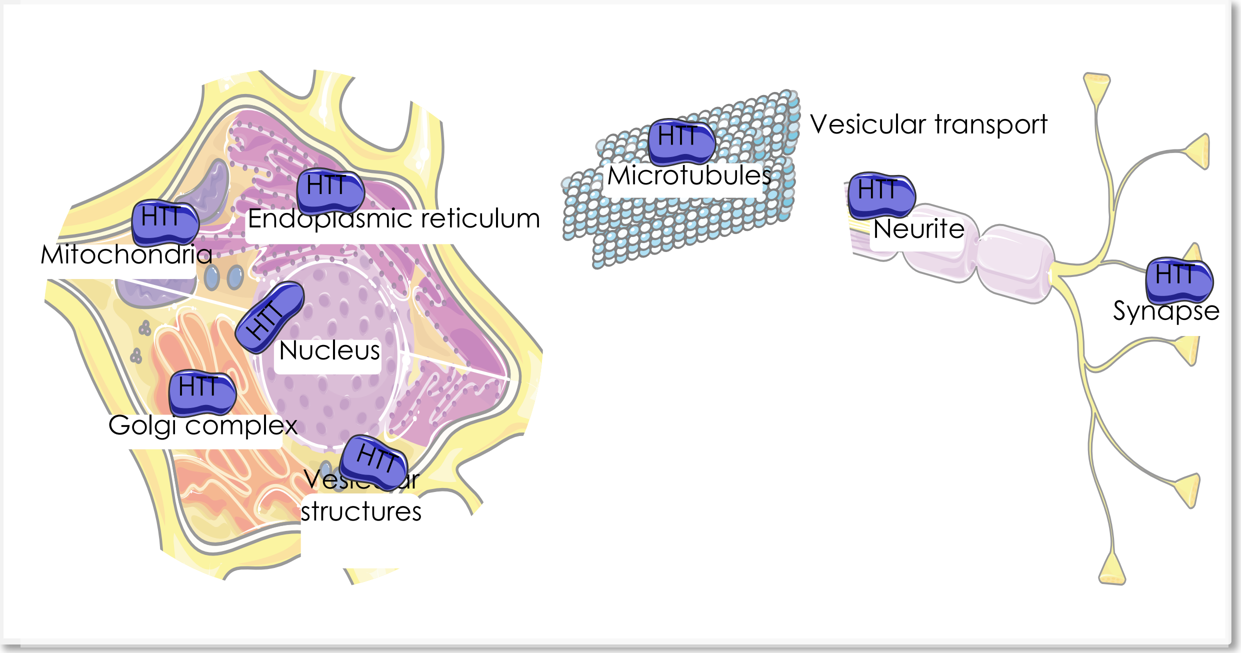
HTT expression in tissues

- HTT is broadly expressed
- However, expression is higher in the nervous system than in other tissues
- Within the brain it is highly expressed in cerebral cortex



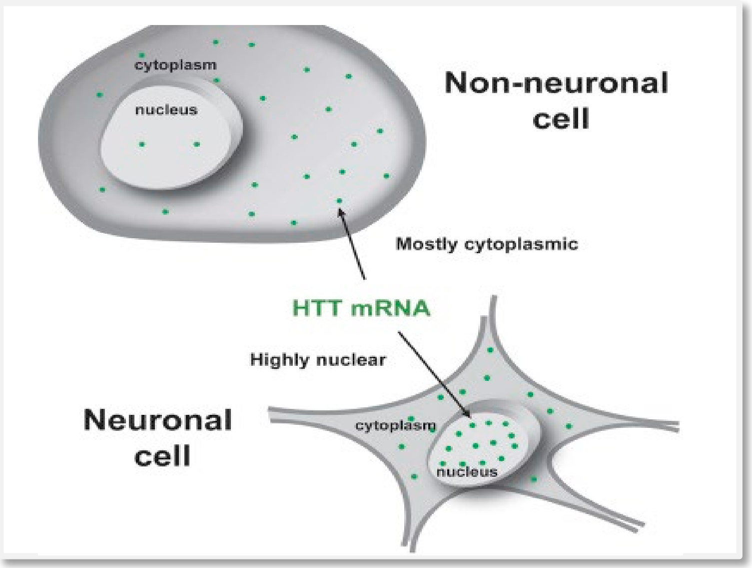
HTT sub-cellular localization

Hilditch-Maguire et al., H M Gen 2000
Hoffner et al., J Cell Sci 2002
Kegel et al., J Biol Chem 2002
Panov et al., Nat Neurosci 2002
Strehlow et al., Hum Mol Genet 2007
DiFiglia et al., Neuron 1995
Velier et al., Exp Neurol 1998
Steffan et al., PNAS 2000
Smith et al., Cell Mol Life Sci 2005
Caviston et al., Trends Cell Biol 2009



~50% of wild-type ***HTT* mRNA** localizes to the nucleus

This nuclear localization is observed only in neuronal cells.



Didiot et al., Cell Rep. 2018

Agenda

- Mutant Htt toxicity
- What is the evidence that wtHTT is important?
- Is there loss of wtHTT function in HD?

Agenda

- **Mutant Htt toxicity**
- What is the evidence that wtHTT is important?
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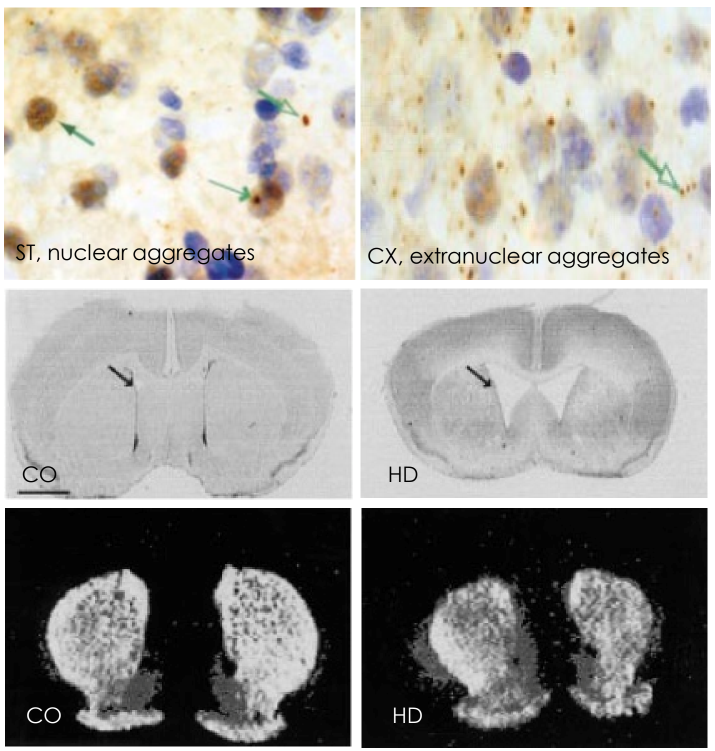
Reversal of Neuropathology and Motor Dysfunction in a Conditional Model of Huntington's Disease

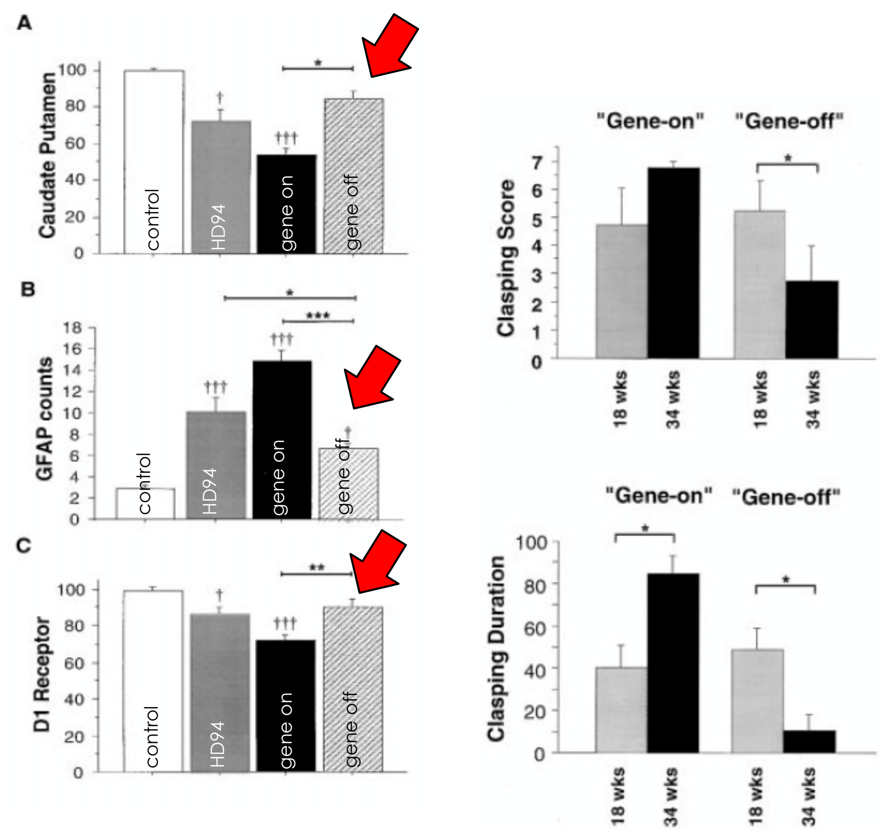
Cell 2000

Ai Yamamoto,† José J. Lucas,†‡ and René Hen*

Turning off muHTT is sufficient to reverse the disease in mice

Neuropathology & motor abnormalities





Early Increase in Extrasynaptic NMDA Receptor Signaling and Expression Contributes to Phenotype Onset in Huntington's Disease Mice

2

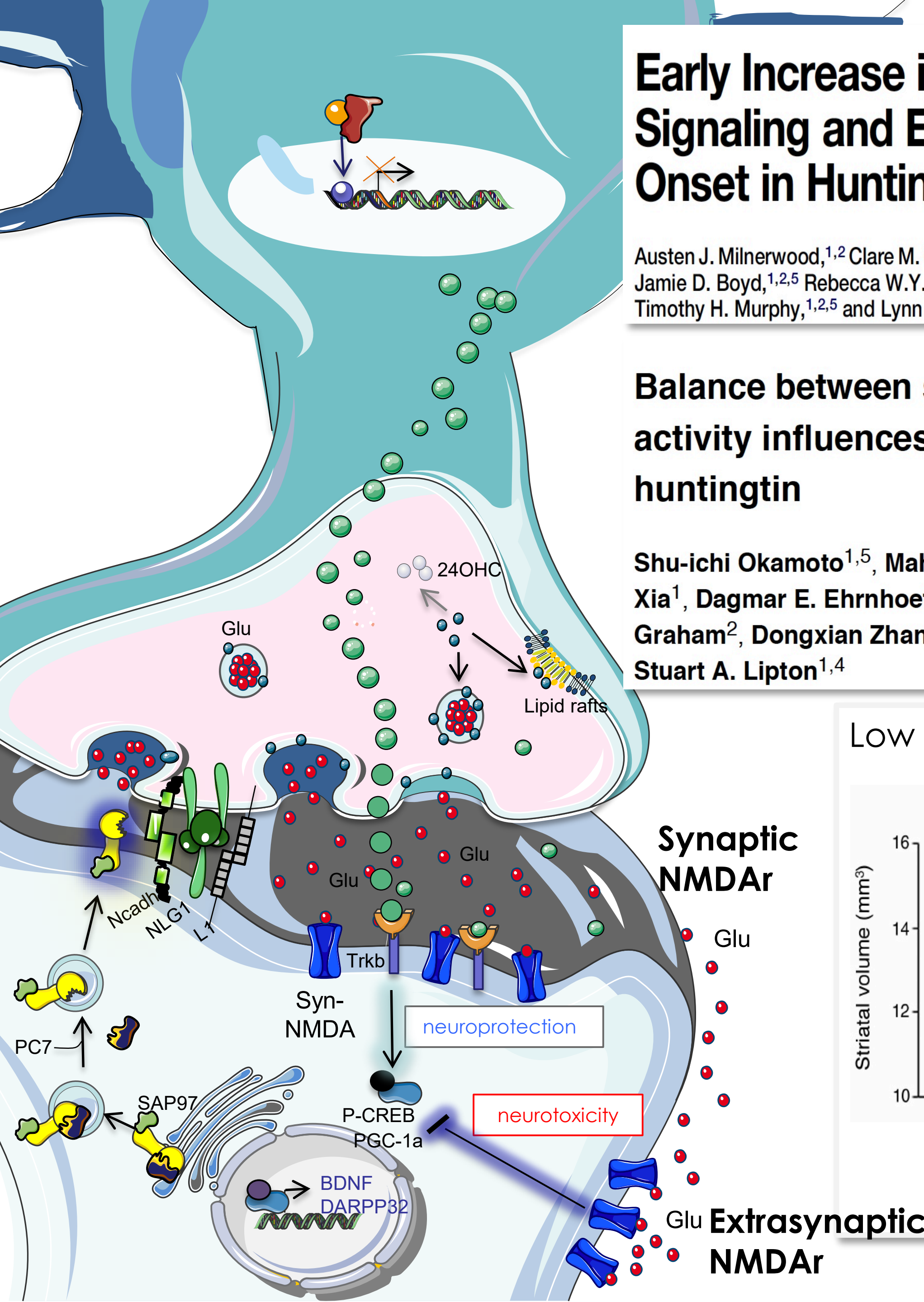
Neuron 2010

Austen J. Milnerwood,[1,2] Clare M. Gladding,[1,2] Mahmoud A. Pouladi,[2,3,4,6] Alexandra M. Kaufman,[1,2] Rochelle M. Hines,[1,2] Jamie D. Boyd,[1,2,5] Rebecca W.Y. Ko,[1,2] Oana C. Vasuta,[1,2] Rona K. Graham,[2,3,4,6] Michael R. Hayden,[2,3,4,6] Timothy H. Murphy,[1,2,5] and Lynn A. Raymond[1,2,*]

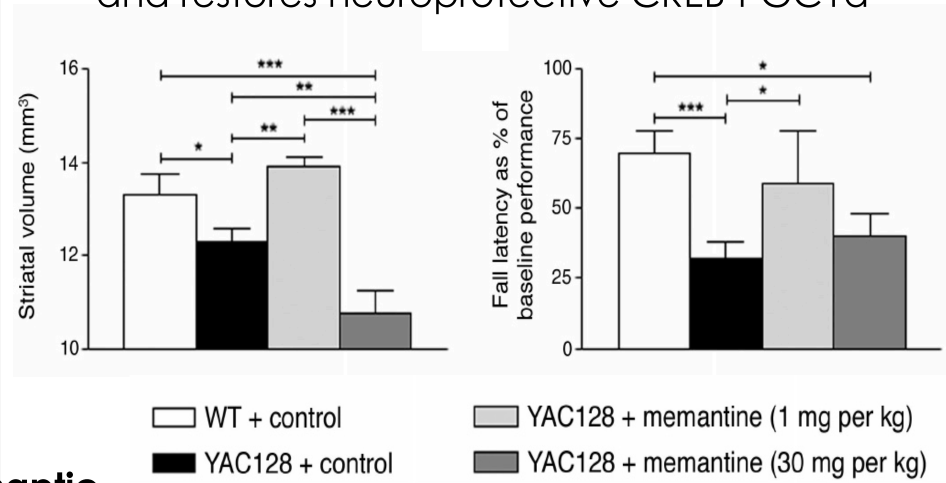
Balance between synaptic versus extrasynaptic NMDA receptor activity influences inclusions and neurotoxicity of mutant huntingtin

Nat Med 2009

Shu-ichi Okamoto[1,5], Mahmoud A. Pouladi[2,5], Maria Talantova[1,5], Dongdong Yao[1,5], Peng Xia[1], Dagmar E. Ehrnhoefer[2], Rameez Zaidi[1], Arjay Clemente[1], Marcus Kaul[1], Rona K. Graham[2], Dongxian Zhang[1], H.-S. Vincent Chen[1,3], Gary Tong[1,4], Michael R. Hayden[2], and Stuart A. Lipton[1,4]



Low conc Memantine blocks extrasynaptic NMDARs and restores neuroprotective CREB-PGC1a



Neuronal targets for reducing mutant huntingtin expression to ameliorate disease in a mouse model of Huntington's disease

nature medicine 2014

Nan Wang[1,2,9], Michelle Gray[1,2,8,9], Xiao-Hong Lu[1,2], Jeffrey P Cantle[1,2], Sandra M Holley[2,3], Erin Greiner[1,2,4], Xiaofeng Gu[1,2], Dyna Shirasaki[1,2,4], Carlos Cepeda[2,3], Yuqing Li[5], Hongwei Dong[6,8], Michael S Levine[2,3] & X William Yang[1,2,7]

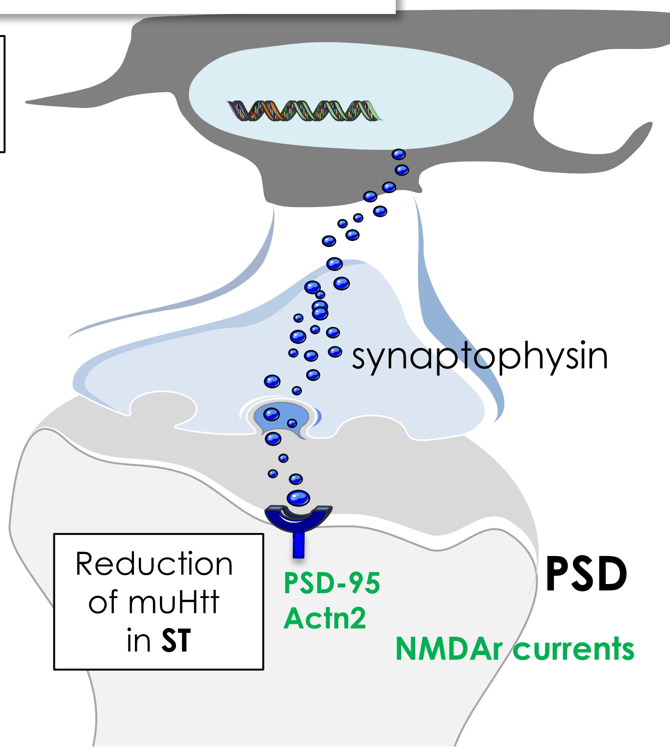
BACHD;
Rgs9-Cre *Tg*
Reduction in striatum

BACHD;
Emx1-Cre *Tg*
Reduction in cortex

BACHD;
Emx1-Cre *Tg*; Rgs9-Cre *Tg*;
Reduction in cortex and striatum

BACHD phenotypes	Reduction of muHtt in **ST**	Reduction of muHtt in **CX**	Reduction of muHtt in **ST+CX**
Motor deficits			
Rotarod (6 months)	NS (29%)	(45%)*	(70%)**
Rotarod (12 months)	NS (19%)	NS (30%)	(63%)*
Spontaneous locomotion (6 months)	NS (39%)	NS (35%)	(82%)*
Spontaneous locomotion (12 months)	NS (30%)	(83%)**	(71%)*
Psychiatric-like behaviors			
Anxiety (light-dark box)	NS (29%)	(85%)***	(100%)***
Depression-like (forced swim test)	NS (12%)	(100%)**	(80%)**
Neurodegeneration			
Forebrain weight	NS (–17%)	NS (32%)	(81%)*
Cortical volume loss	NS (51%)	NS (54%)	(100%)**
Striatal volume loss	NS (36%)	NS (27%)	(100%)**

Percentage of improvement for a given phenotype represents the absolute mean value of the difference between BE, BR, BER and BACHD divided by the absolute mean value of the difference between BACHD and WT. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.



Cell autonomous toxicity of muHTT in striatum

Neuronal targets for reducing mutant huntingtin expression to ameliorate disease in a mouse model of Huntington's disease

nature medicine 2014

Nan Wang[1,2,9], Michelle Gray[1,2,8,9], Xiao-Hong Lu[1,2], Jeffrey P Cantle[1,2], Sandra M Holley[2,3], Erin Greiner[1,2,4], Xiaofeng Gu[1,2], Dyna Shirasaki[1,2,4], Carlos Cepeda[2,3], Yuqing Li[5], Hongwei Dong[6,8], Michael S Levine[2,3] & X William Yang[1,2,7]

BACHD;
Rgs9-Cre *Tg*
Reduction in striatum

BACHD;
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Reduction in cortex

BACHD;
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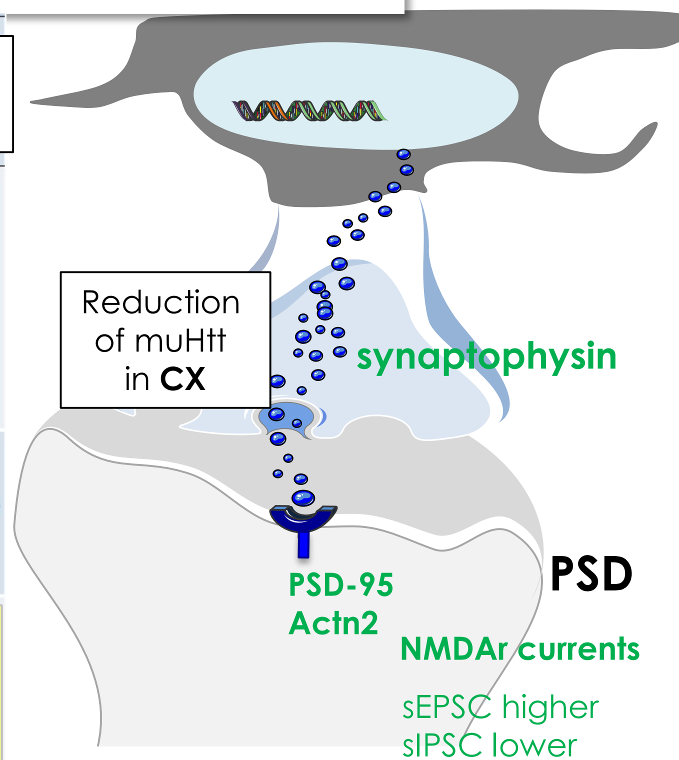
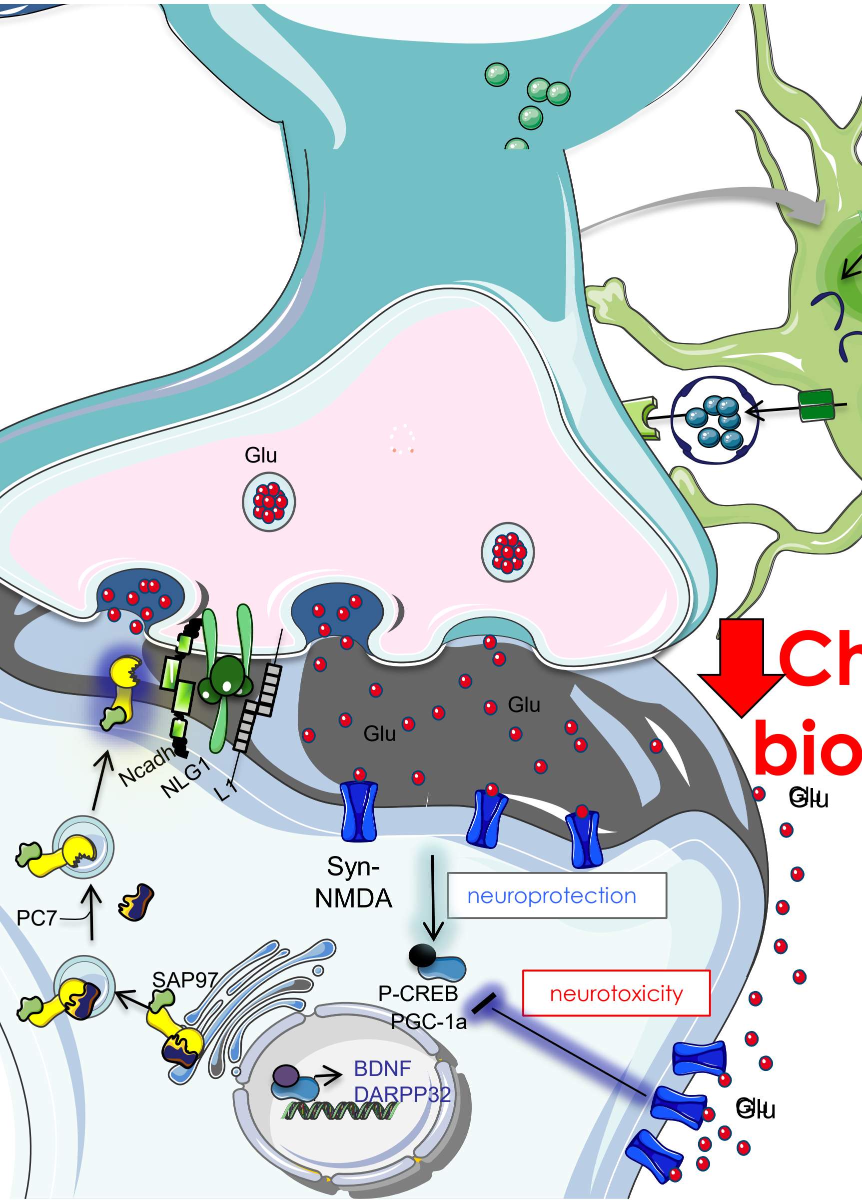
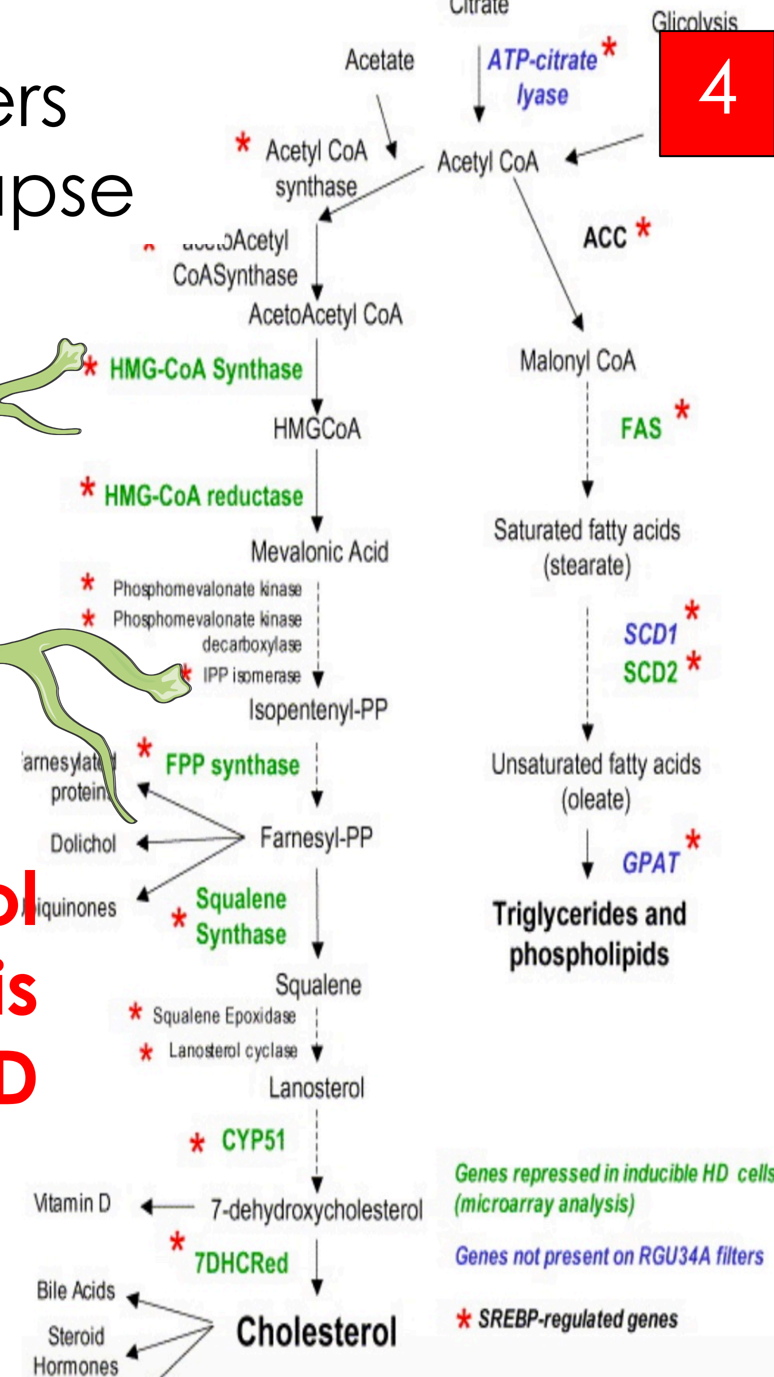


Non-cell autonomous toxicity of muHTT in striatum

Astrocytes, additional players at the HD cortico-striatal synapse

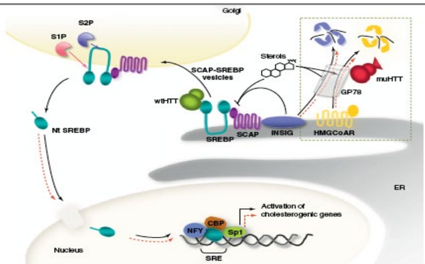


Cholesterol biosynthesis In HD



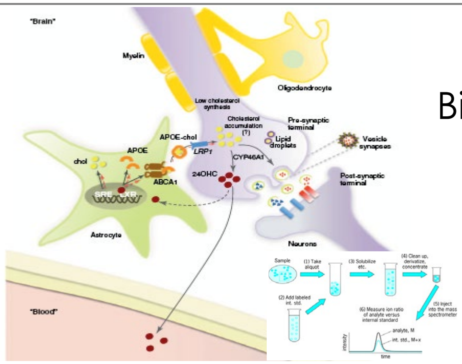
Sipione, Hum Mol Gen 2002

Astrocytes, additional players at the HD cortico-striatal synapse



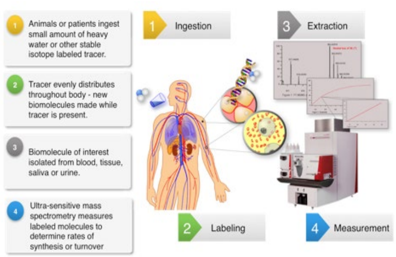
Molecular mechanisms

2005-2015
Valenza, J. Neurosci 2005
Bobrowska, PlosOne 2012
Lee, Neuron 2014
Valenza, Cell Death Differ 2015



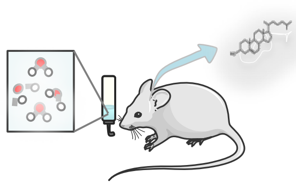
Biological relevance

2007-2017
Trushina, Hum Mol Gen 2006
Valenza, Hum Mol Gen 2007
Valenza, Neurobiol Dis 2007
Futter, J. Med gen 2009
Valenza, Neurosci 2010
Luthi-Carter, PNAS 2010
Del Toro, J. Neurochem 2010
Xiang, J. Neurosci 2011
Koga, J. Neurosci 2011
Marullo, PlosHD 2012
Ritch, Mol Cell Neurosci. 2012
Trushina, Hum Mol Gen 2013
Gao, Biochemistry 2015
Kreilus, Journal of HD 2015
Shankaran, Neurobiol Dis 2017
Boussicault, Biochemie, 2018



24OH as a Biomarker

2008-2013
Leoni, Brain 2008
Leoni, Neurosci Lett 2011
Leoni, Neurobiol Dis 2013
Mariotti, Longitudinal studies on-going



Therapeutical strategies
(nanoparticles mediated cholesterol delivery; CYP46 gene therapy)

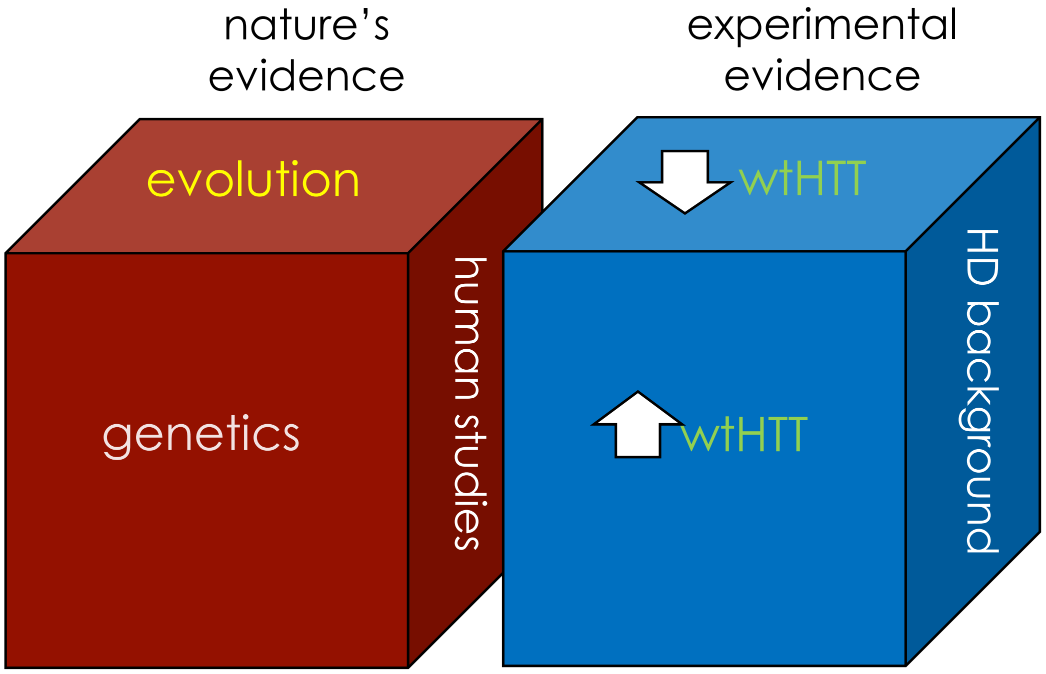
2015-2019
Valenza, EMBO Mol Med, 2015
Manuscript in preparation (Cattaneo Lab)
Boussicault, Brain, 2016
Kacher, Brain, 2019

Conclusions

- muHTT toxicity causes nuclear, cytoplasmic and mitochondrial pathology; it affects **neurons** and **astrocytes;** it acts in a cell autonomous and **non-cell** autonomous manner; **toxicity can be reversed** by turning off muHTT expression; in a disease-modifying therapy **both striatum and cortex** should be targeted.

Agenda

- Mutant Htt toxicity
- **What is the evidence that wtHTT is important?**
- Is there loss of wtHTT function in HD?



nature's evidence
experimental evidence
evolution
genetics
human studies
wtHTT
wtHTT
HD background

1

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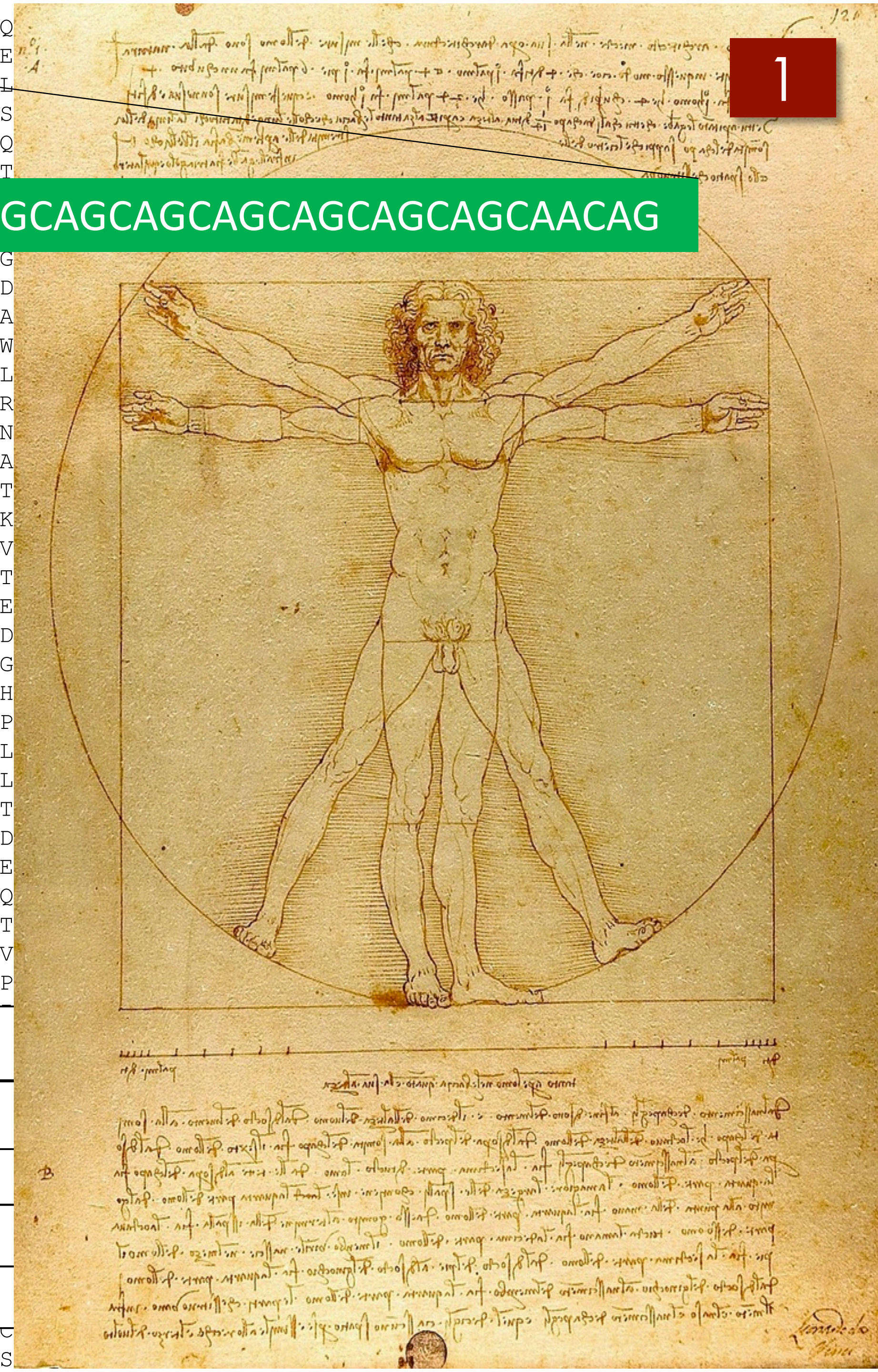
Human HTT
chr4
67 exons
3144 aa
340 KDa



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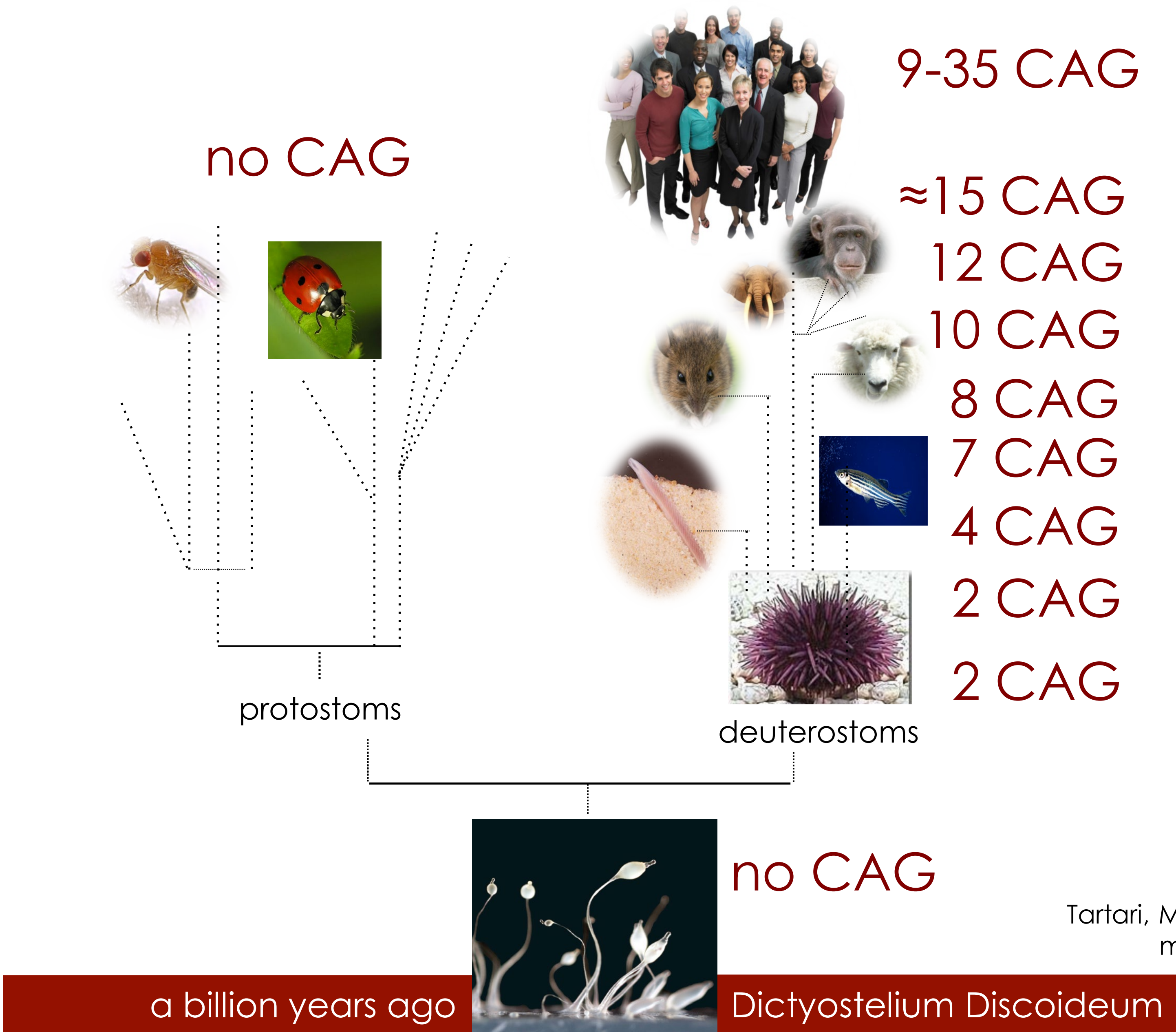
Dicty HTT
chr2
4 exons
3095 aa
330 KDa

Myre, PLOS Genetics, 2011

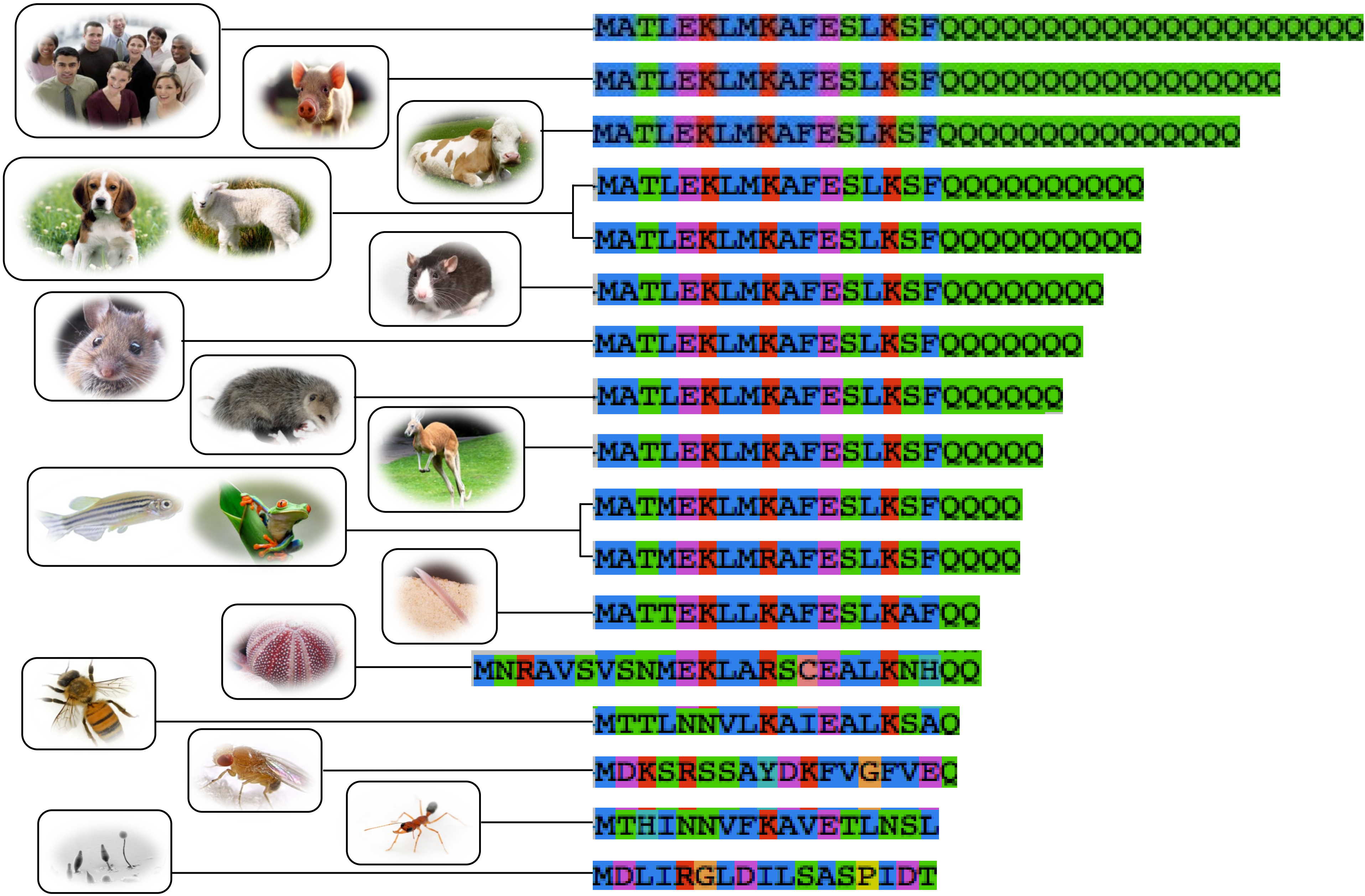
HTT CAG along the human lineage

1



Tartari, Mol. Biol. Evol, 2008;
more work ongoing

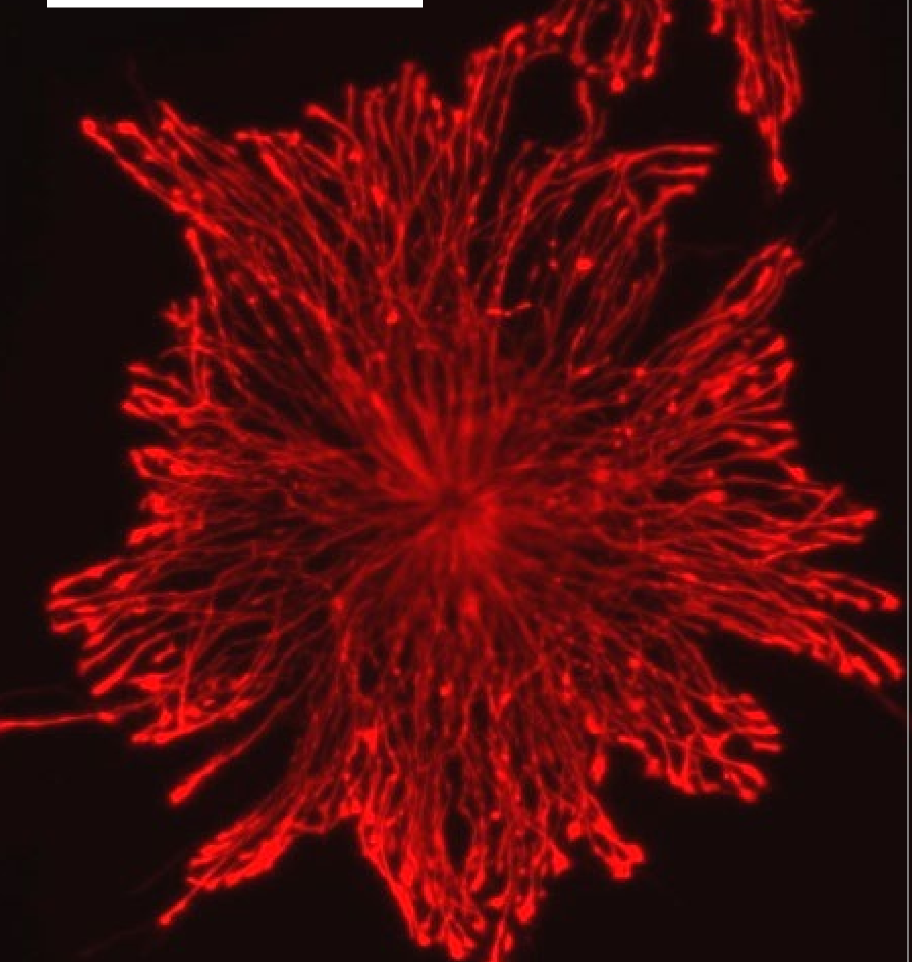
HTT polyQ along the human lineage



An evolutionary recent neuroepithelial cell adhesion function of huntingtin implicates ADAM10-Ncadherin

Valentina Lo Sardo, Chiara Zuccato, Germano Gaudenzi, Barbara Vitali, Catarina Ramos, Marzia Tartari, Michael A Myre, James A Walker, Anna Pistocchi, Luciano Conti, Marta Valenza, Binia Drung, Boris Schmidt, James Gusella, Scott Zeitlin, Franco Cotelli & Elena Cattaneo

nature neuroscience 2012



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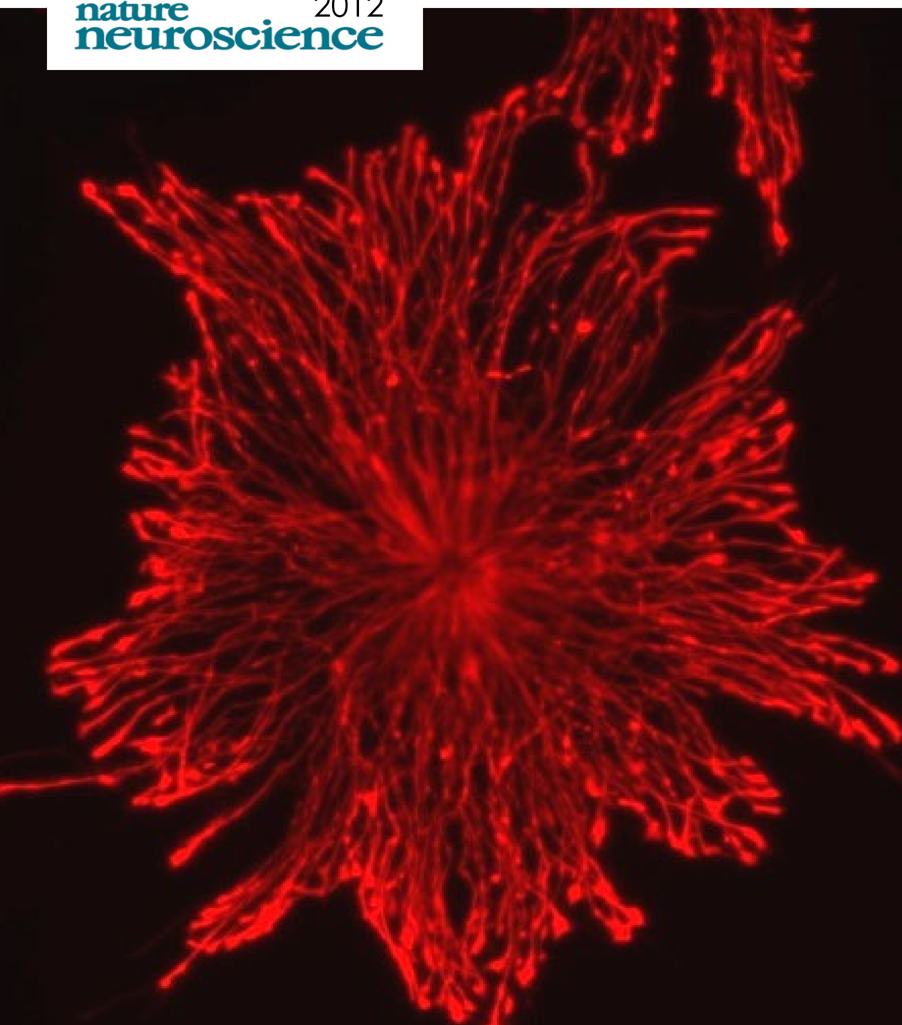

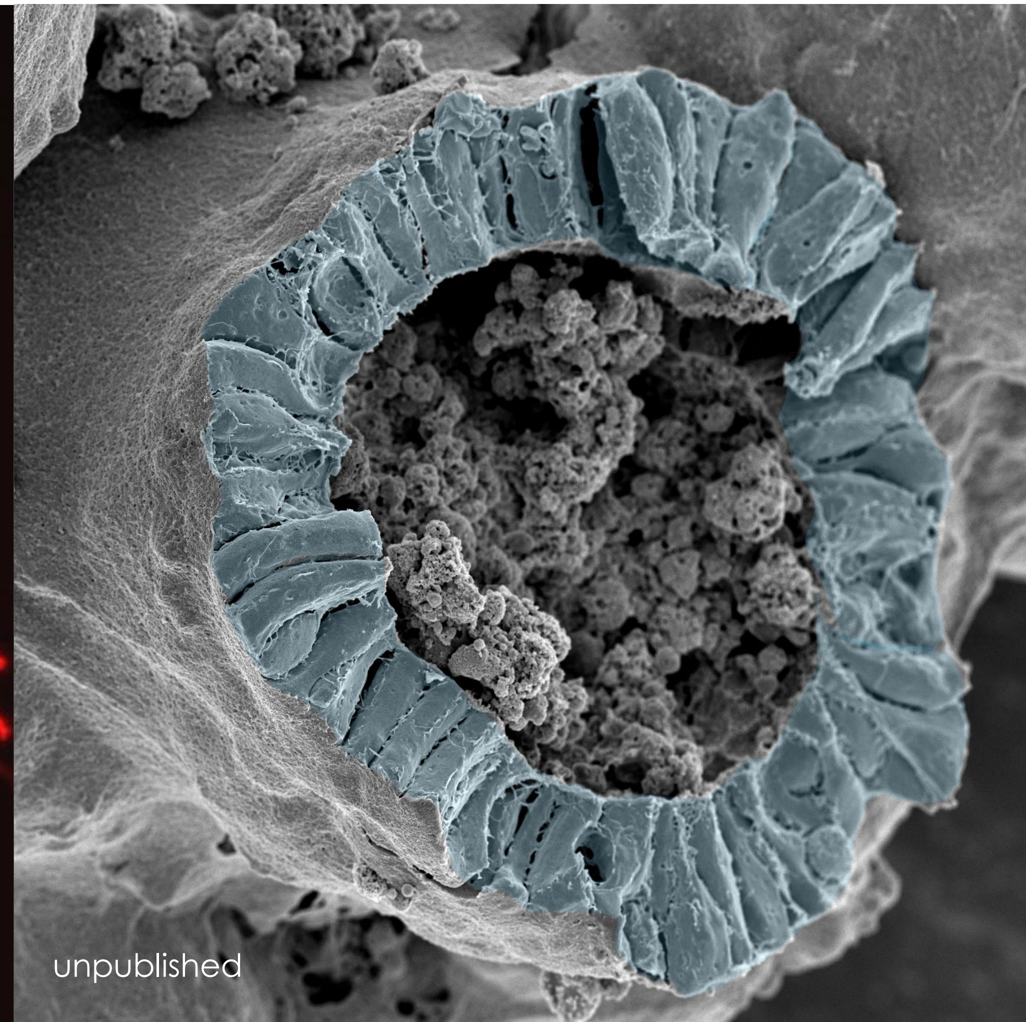

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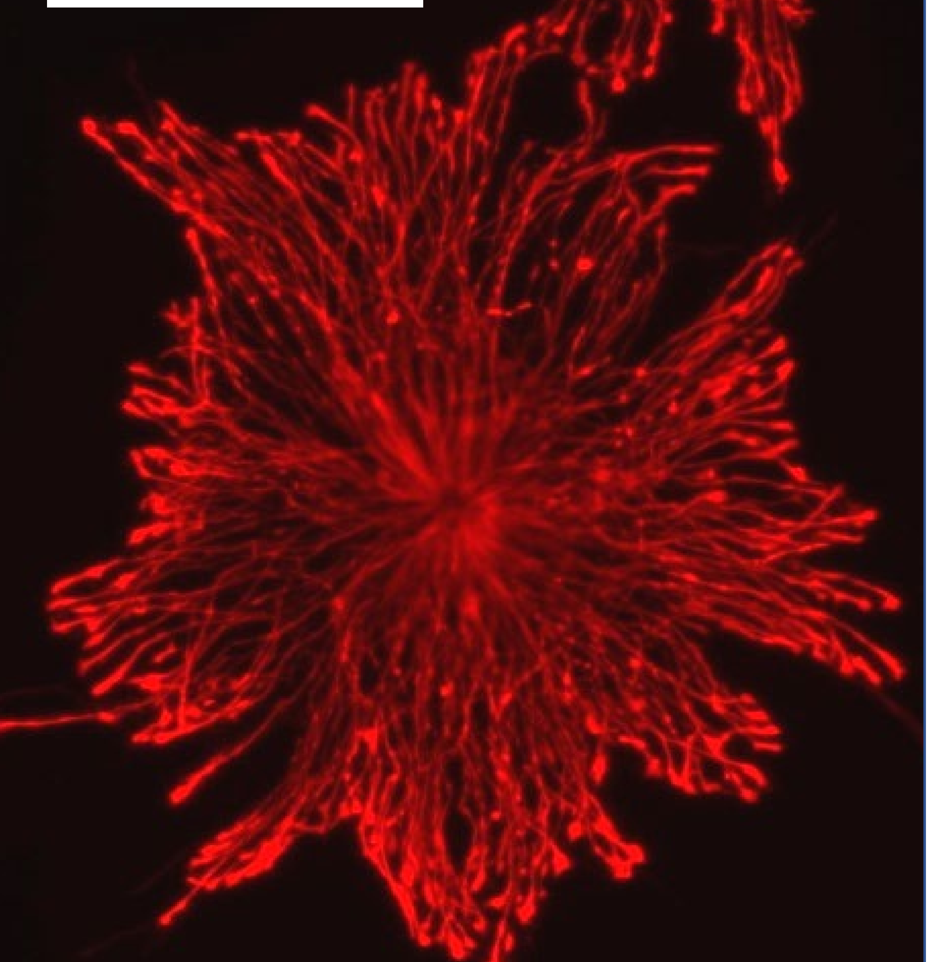
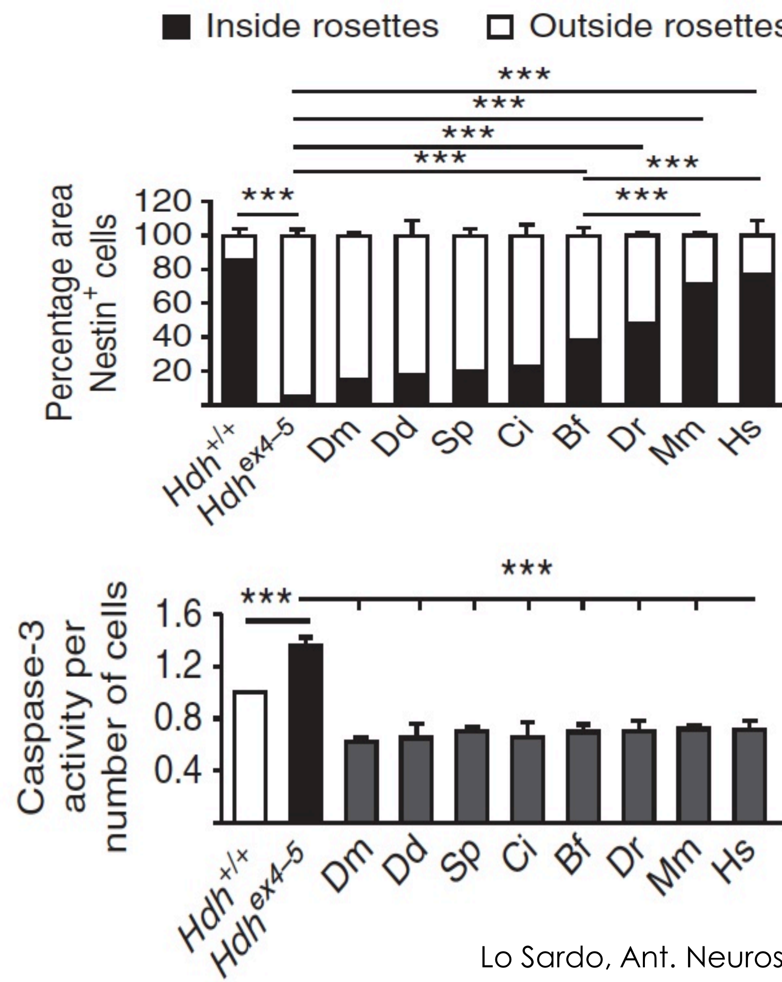


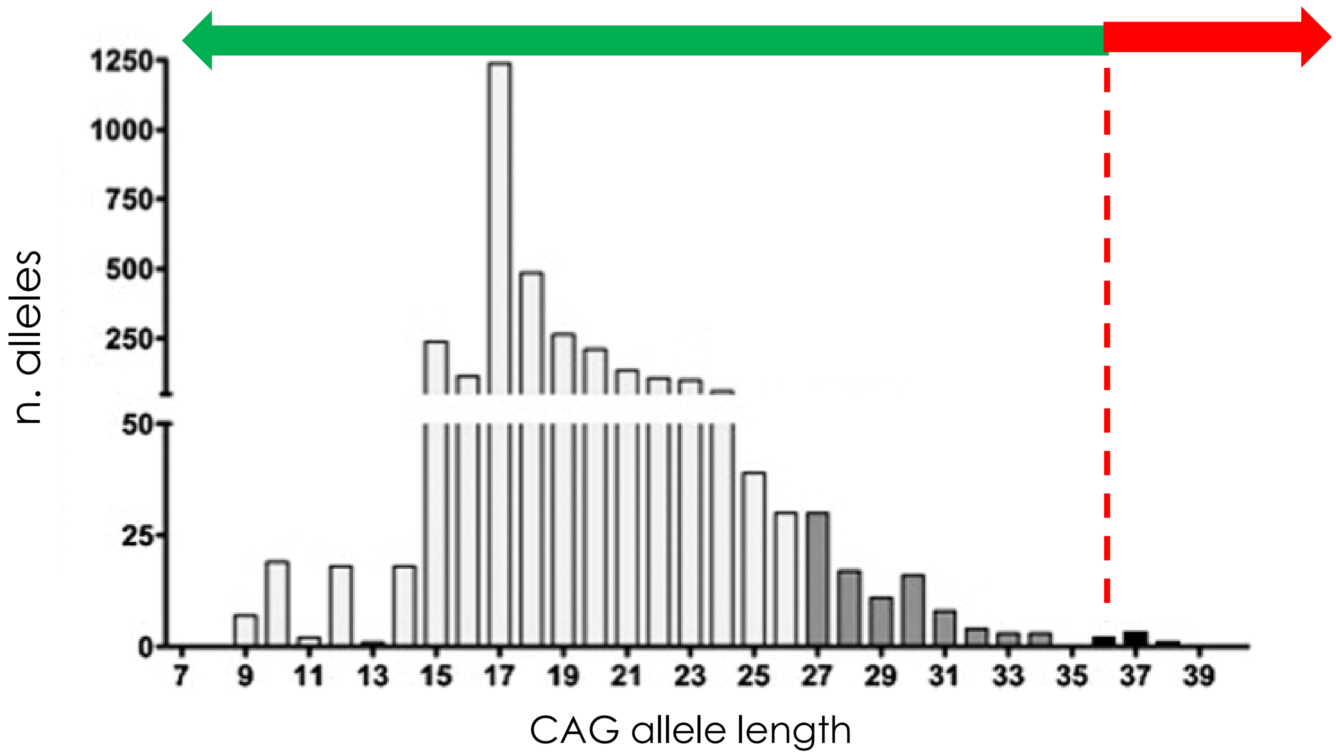


Lo Sardo, Ant. Neurosci 2012

CAG size-specific risk estimates for intermediate allele repeat instability in Huntington disease

Alicia Semaka,[1] Chris Kay,[1] Crystal Doty,[1] Jennifer A Collins,[1] Emilia K Bijlsma,[2] Fiona Richards,[3] Y Paul Goldberg,[1,4] Michael R Hayden[1]

J. Med. Gen, 2013



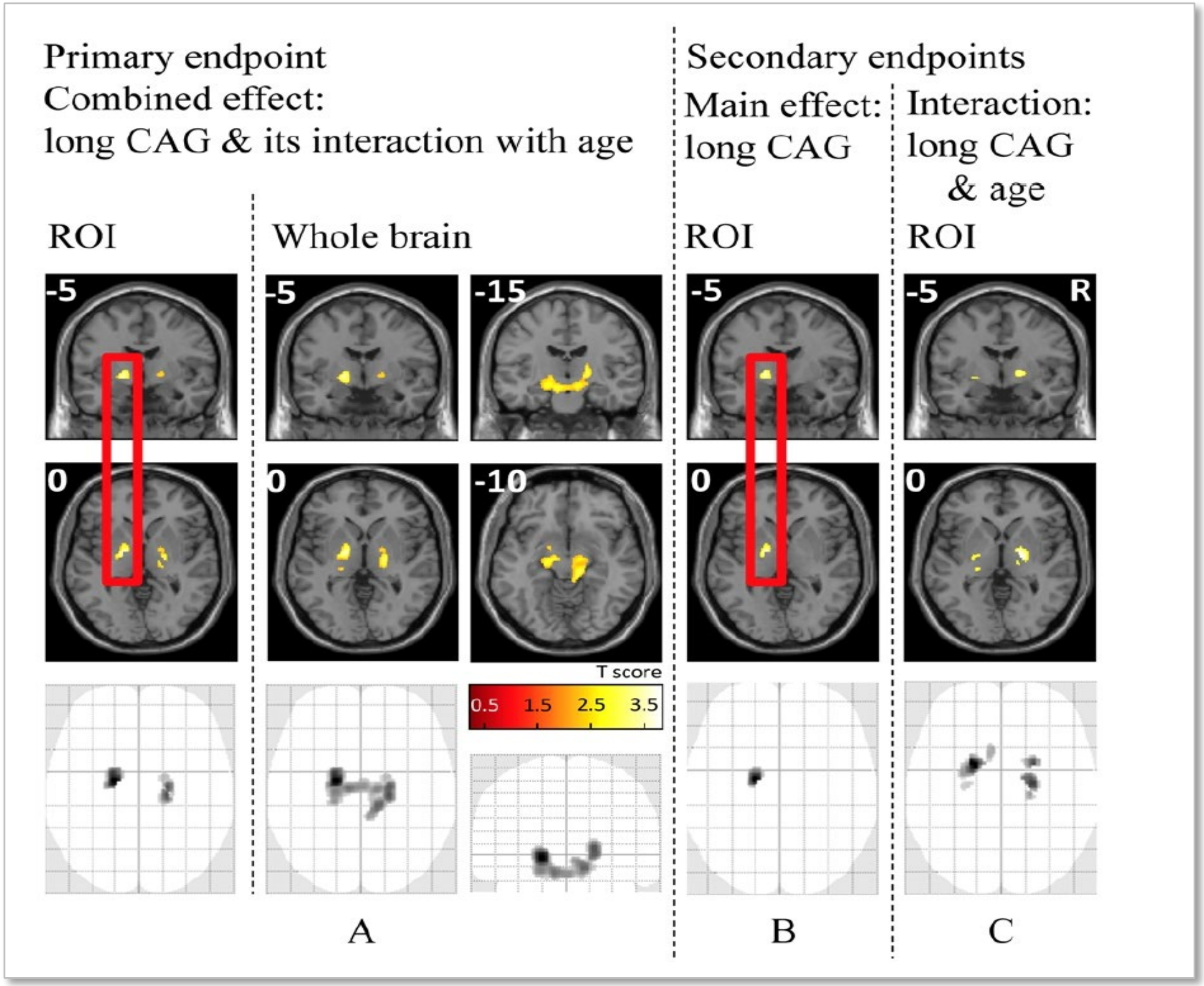
1/17 individuals carries an IA (27-35 CAG) = 5.8%

also see Rubinsztein, Nature Genetics, 1994

Variation within the Huntington's Disease Gene Influences Normal Brain Structure

Plos One, 2012

Mark Mühlau[1*], Juliane Winkelmann[1,2,3], Dan Rujescu[4], Ina Giegling[4], Nikolaos Koutsouleris[4], Christian Gaser[5], Milan Arsic[1], Adolph Weindl[1], Maximilian Reiser[6], Eva M. Meisenzahl[4]

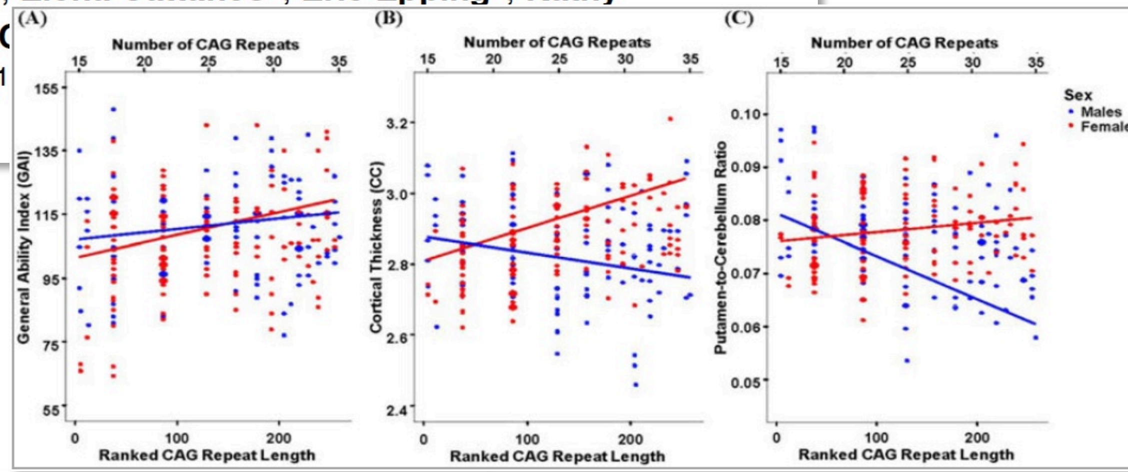


MRI scan on 278 subjects

JNR, 2017

Sex-Specific Effects of the Huntington Gene on Normal Neurodevelopment

Jessica K. Lee[1], Yue Ding[1], Amy L. Conrad[2], Elena Cattaneo[3], Eric Epping[1], Kathy Mathews[2,4], Pedro Gonzalez-Alegre[5], Larry C
Schlaggar[8,9,10,11,12], Joel S. Perlmutter[8,10,11
Peg Nopoulos[1,2,4,*]



Effect of Trinucleotide Repeats in the Huntington's Gene on Intelligence

Jessica K. Lee [a], Amy Conrad [b], Eric Epping [a], Kathy Mathews [b,c], Vincent Magnotta [d], Jeffrey D. Dawson [e], Peg Nopoulos [a,b,c,*]

EBioMedicine, 2018

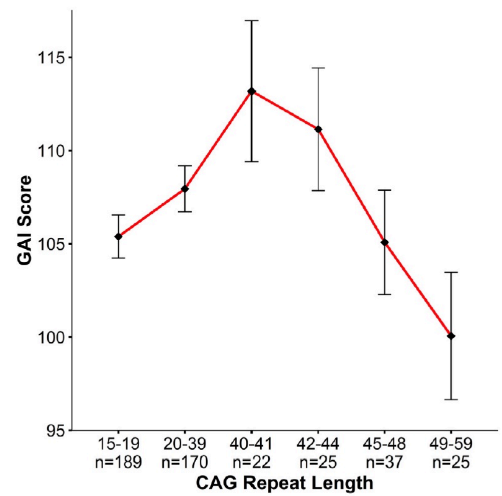
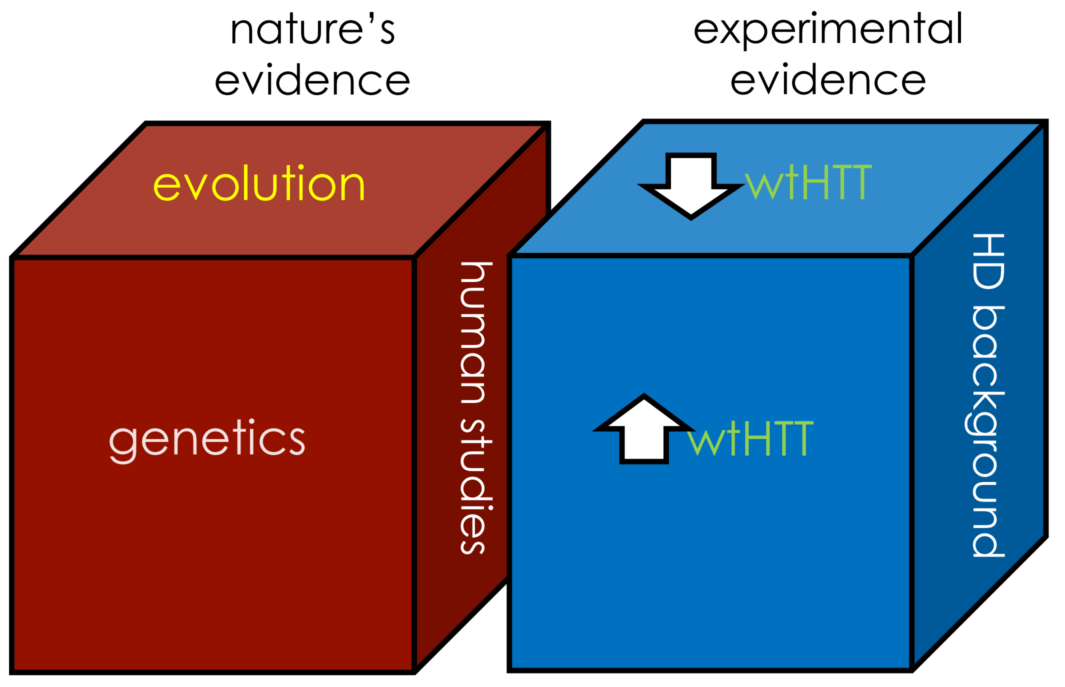


Fig. 2. General abilities index (GAI). Graph above shows results of the non-linear model ($\beta = -20.2$, $p = .006$) where the x-axis is represented by groups of subjects binned by CAG repeat length of the longest allele, and the y-axis is the mean GAI (bars are standard error) for each group. To obtain mean GAI, ANCOVA was performed between groups, controlling for age, sex, and parental SES.

Conclusions

- muHTT toxicity causes nuclear, cytoplasmic and mitochondrial pathology; it affects **neurons** and **astrocytes;** it acts in a cell autonomous and **non-cell** autonomous manner; **toxicity can be reversed** by turning off muHTT expression; in a disease-modifying therapy **both striatum and cortex** should be targeted.

- for a **billion years** nature **has not** eliminated huntingtin but implemented its functions by lengthening its CAG



nature's evidence
experimental evidence
evolution
genetics
human studies
wtHTT
wtHTT
HD background

wtHTT is important during development

- Knockout is embryonic lethal
- Heterozygote demonstrates neurodegeneration in subthalatic nucleus and globus pallidus
- Postnatal Htt reduction detrimental
- Mutant Htt retains normal function

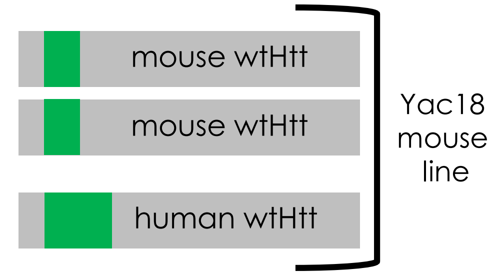
What is the evidence that **wtHTT continues to be required throughout life** to support neuronal survival and homeostasis?

What happens **if we reduce wtHTT** level in the adult brain under stressed conditions (HD)?

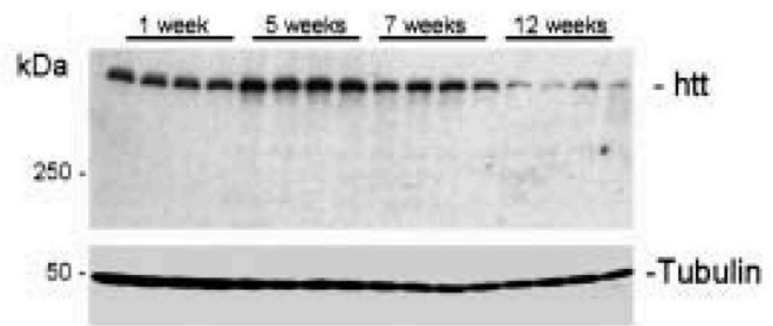
Depletion of wild-type huntingtin in mouse models of neurologic diseases

J. of Neurochem 2003

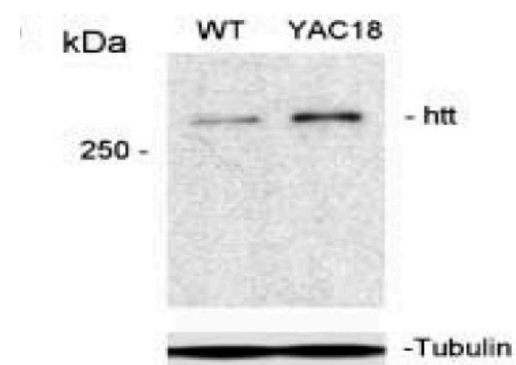
Yu Zhang,* Mingwei Li,* Martin Drozda,* Minghua Chen,* Shengjun Ren,† Rene O. Mejia Sanchez,* Blair R. Leavitt,‡ Elena Cattaneo,§ Robert J. Ferrante,¶,** Michael R. Hayden‡ and Robert M. Friedlander*



Depletion of huntingtin in neurologic disease models featuring caspase activation



Wild-type huntingtin overexpression **reduces the lesion volume** after post-ischemic injury



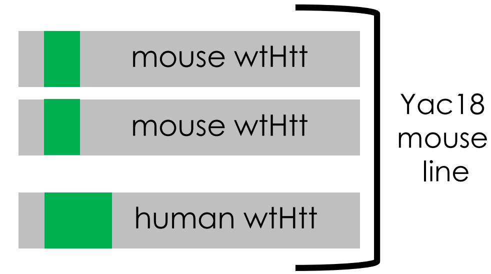
Reduction of post-ischemic injury in YAC18 mice

	WT	YAC18
Lesion volume (% hemisphere)	52.6 ± 3.6	43.9 ± 3.3*
Reduction of lesion volume		17%

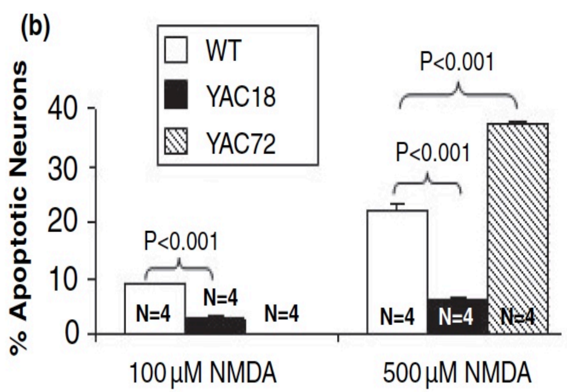
Wild-type huntingtin protects neurons from excitotoxicity

Blair R. Leavitt,*,† Jeremy M. van Raamsdonk,* Jacqueline Shehadeh,‡ Herman Fernandes,‡ Zoe Murphy,* Rona K. Graham,* Cheryl L. Wellington,§ Lynn A. Raymond,†,‡ and Michael R. Hayden,*,†

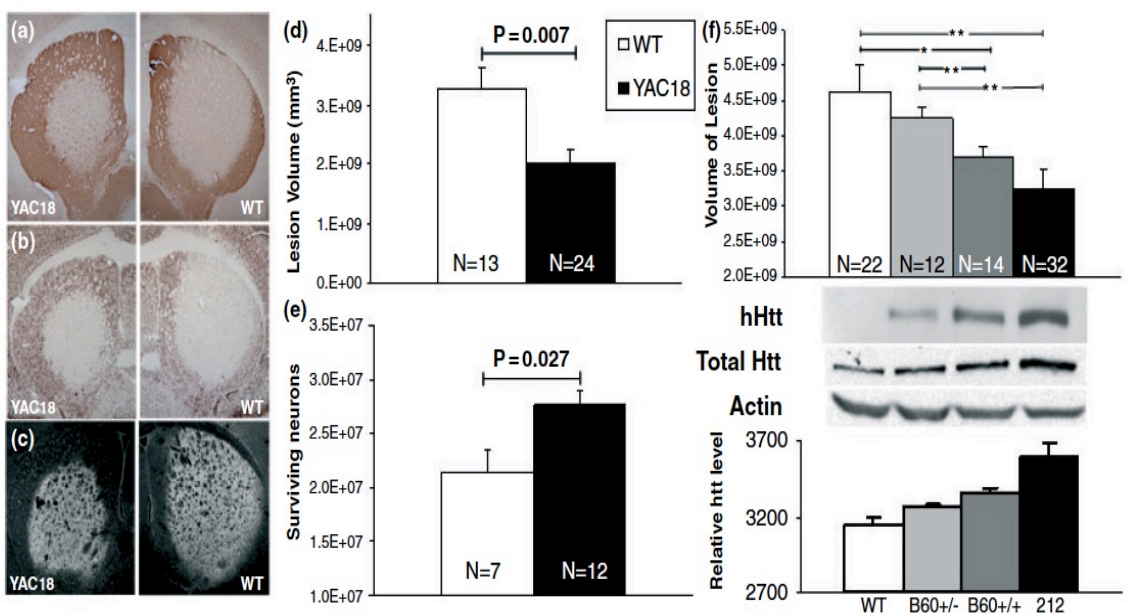
J. of Neurochem 2006



wtHtt is **neuroprotective** in vitro



Over-expression of **wtHtt** in YAC18 mice causes **decreased neurodegeneration** after QA in a dose-dependent manner



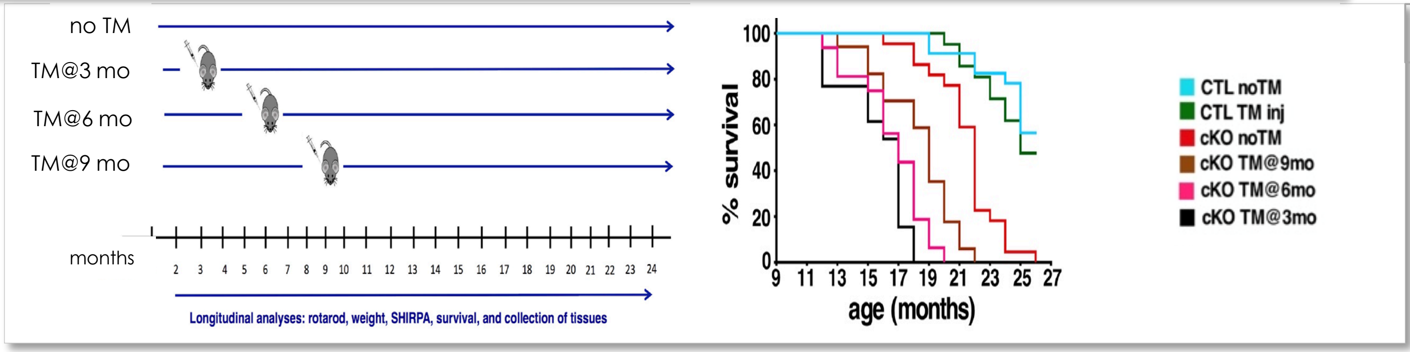
Elimination of huntingtin in the adult mouse leads to progressive behavioral deficits, bilateral thalamic calcification, and altered brain iron homeostasis

Plos Genetics, 2017

Paula Dietrich, Irudayam Maria Johnson[¤a], Shanta Alli[¤b], Ioannis Dragatsis*

3

Long term global HTT loss



Reduced longevity

Elimination of huntingtin in the adult mouse leads to progressive behavioral deficits, bilateral thalamic calcification, and altered brain iron homeostasis

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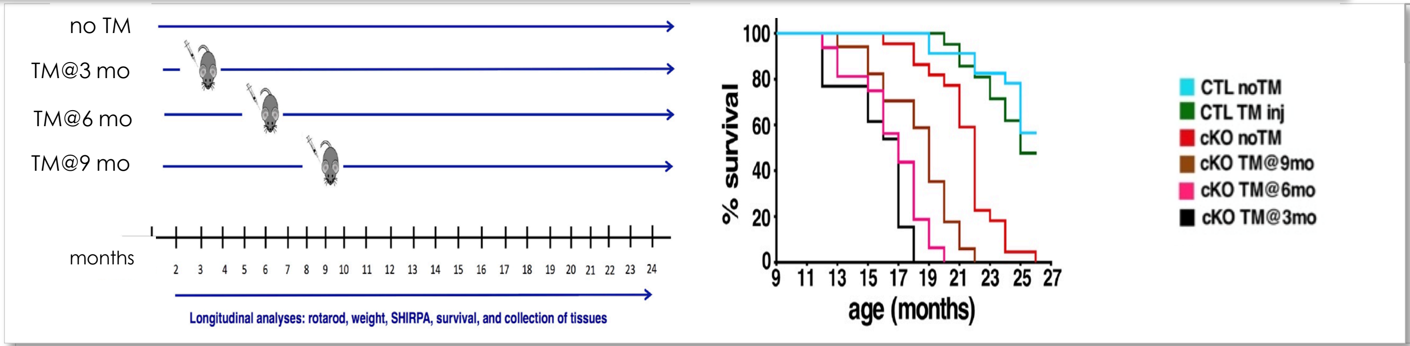
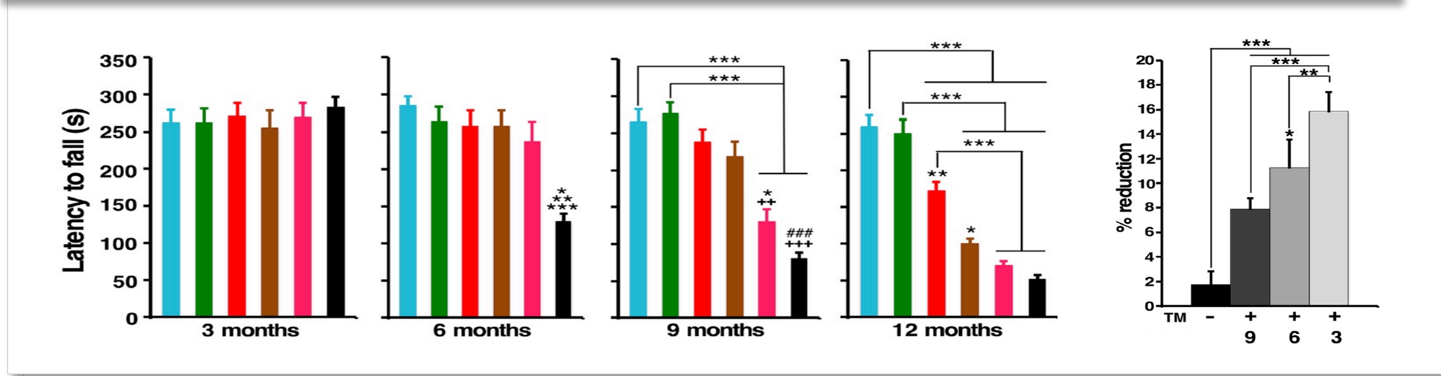
Long term global HTT loss



Reduced longevity



Motor and behavioral deficit

Brain atrophy

Calcification

Iron depletion

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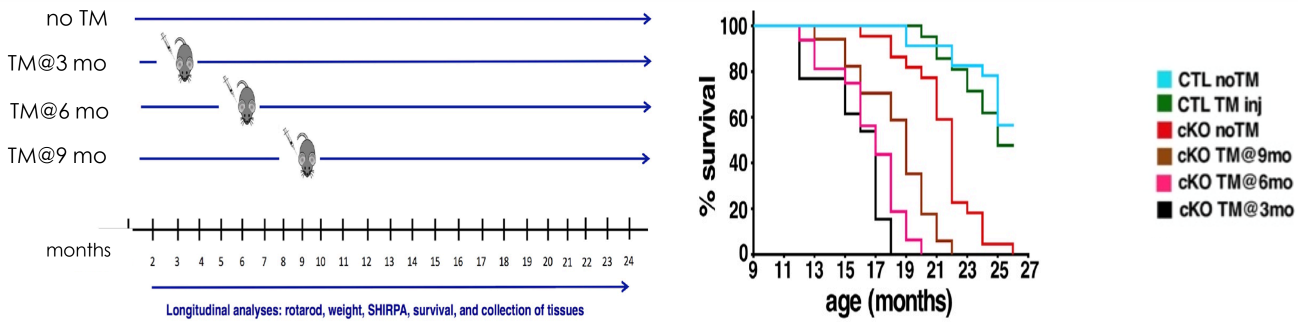
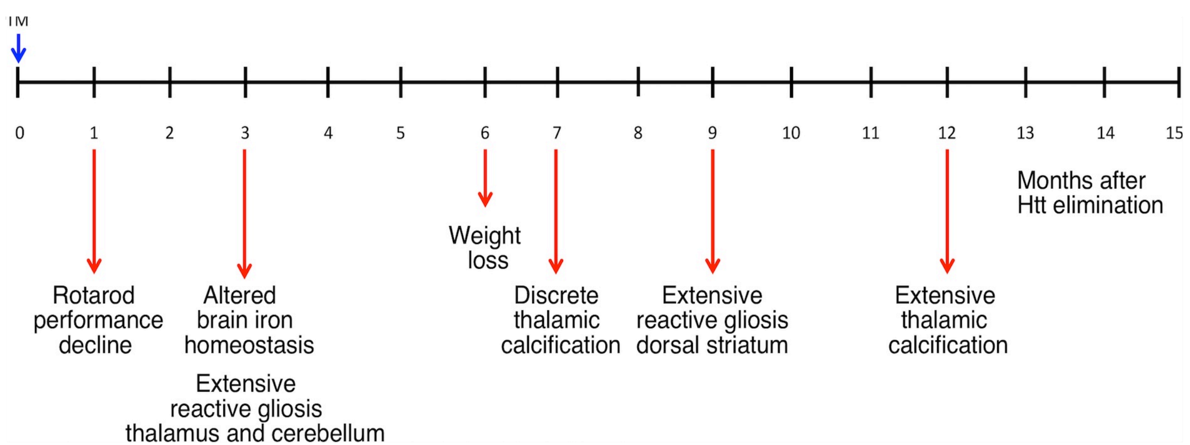


Defects are time dependent

wtHtt elimination causes the same **time-dependent defects** regardless of the stage at which the animals were treated

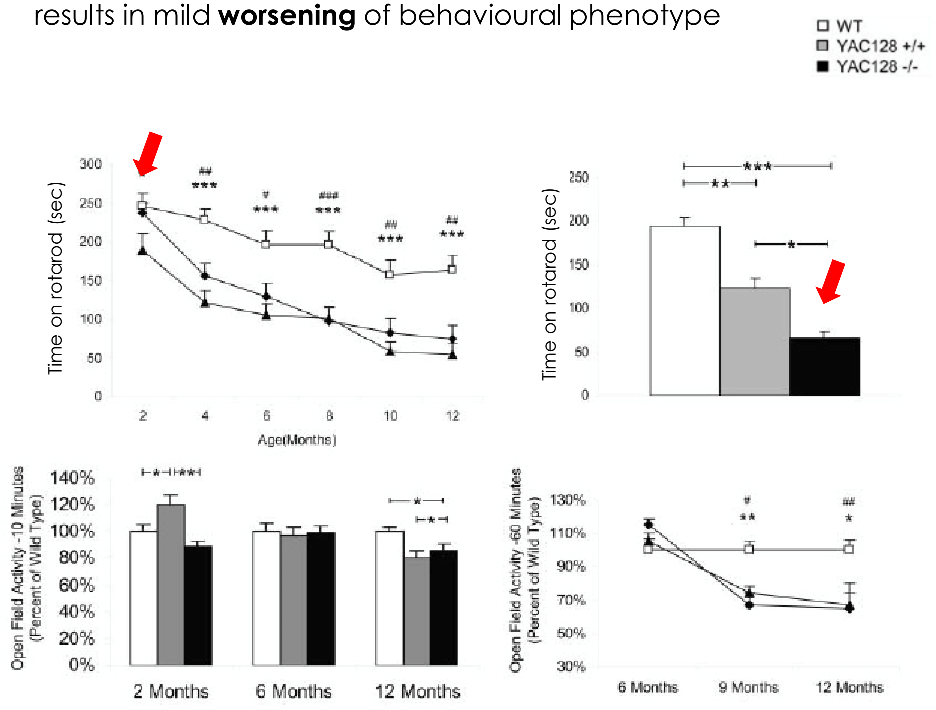


Loss of wild-type huntingtin influences motor dysfunction and survival in the YAC128 mouse model of Huntington disease

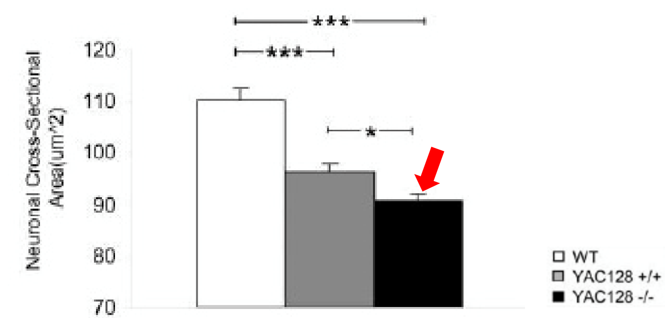
HMG, 2005

Jeremy M. Van Raamsdonk[1,3], Jacqueline Pearson[1,3], Daniel A. Rogers[1,3], Nagat Bissada[1,3], A. Wayne Vogl[2], Michael R. Hayden[1,3,*] and Blair R. Leavitt[1,3]

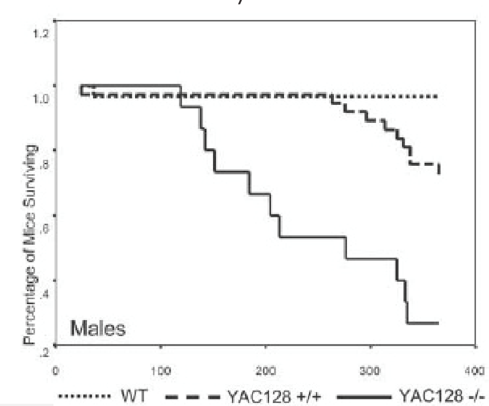
Loss of wild-type htt expression in YAC128 mice results in mild **worsening** of behavioural phenotype



Loss of wild-type htt has a **modest effect** on striatal neuropathology in YAC128 mice



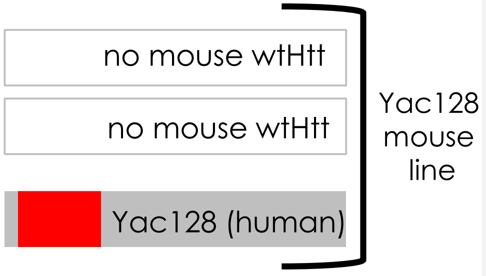
YAC128 mice show a male specific **survival deficit** that is exacerbated by the loss of wthtt expression



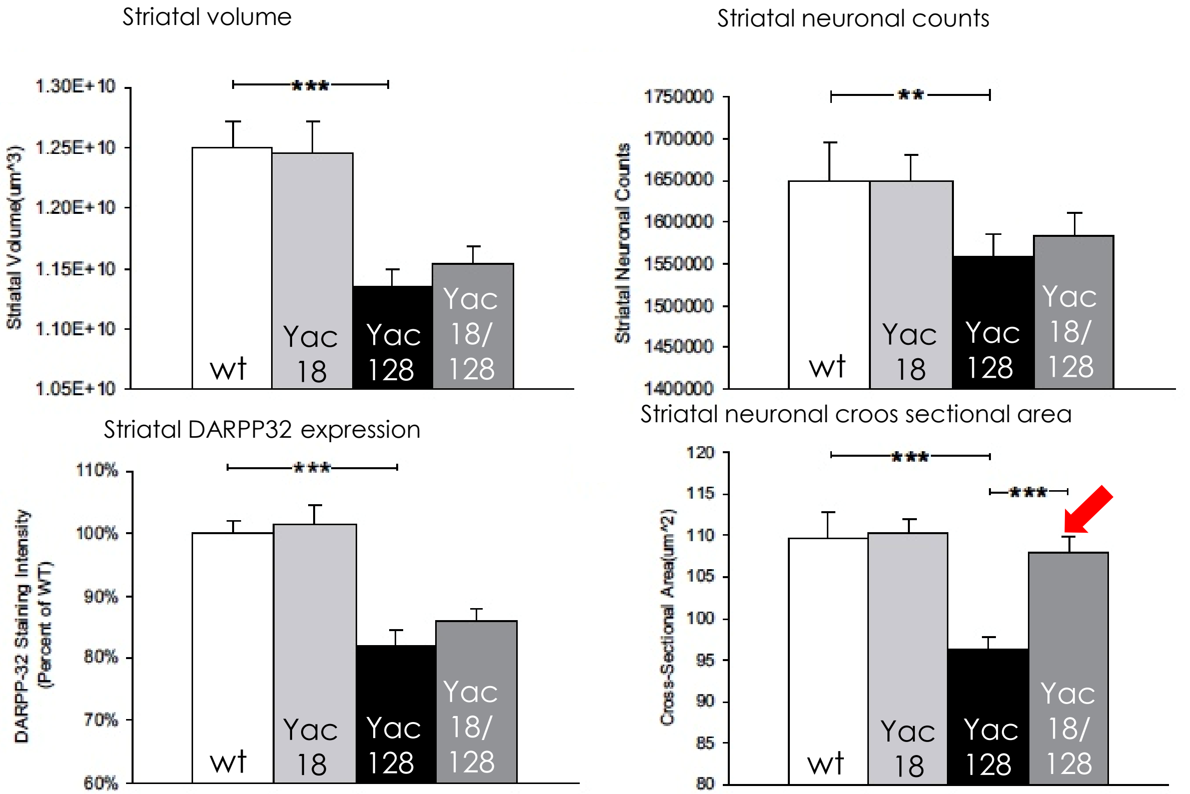
Wild-type huntingtin ameliorates striatal neuronal atrophy but does not prevent other abnormalities in the YAC128 mouse model of Huntington disease

Jeremy M Van Raamsdonk, Jacqueline Pearson, Zoe Murphy, Michael R Hayden* and Blair R Leavitt

BMC Neuroscience 2006



Over-expression of wild-type HTT results in **mild improvements** in striatal neuropathology in YAC128 mice



Conclusions

- muHTT toxicity causes nuclear, cytoplasmic and mitochondrial pathology; it affects **neurons** and **astrocytes;** it acts in a cell autonomous and **non-cell** autonomous manner; **toxicity can be reversed** by turning off muHTT expression; in a disease-modifying therapy **both striatum and cortex** should be targeted.

- for a **billion years** nature **has not** eliminated huntingtin but implemented its functions by lengthening its CAG

- HTT exerts different functions in the developing and adult brain

- In the adult brain wtHTT continues to be necessary and is **neuroprotective** while muHTT is unable to support, for example, BDNF production.

Agenda

- Mutant Htt toxicity
- What is the evidence that wtHTT is important?
- **Is there loss of wtHTT function in HD?**

wtHTT is antiapoptotic while muHtt increases apoptosis

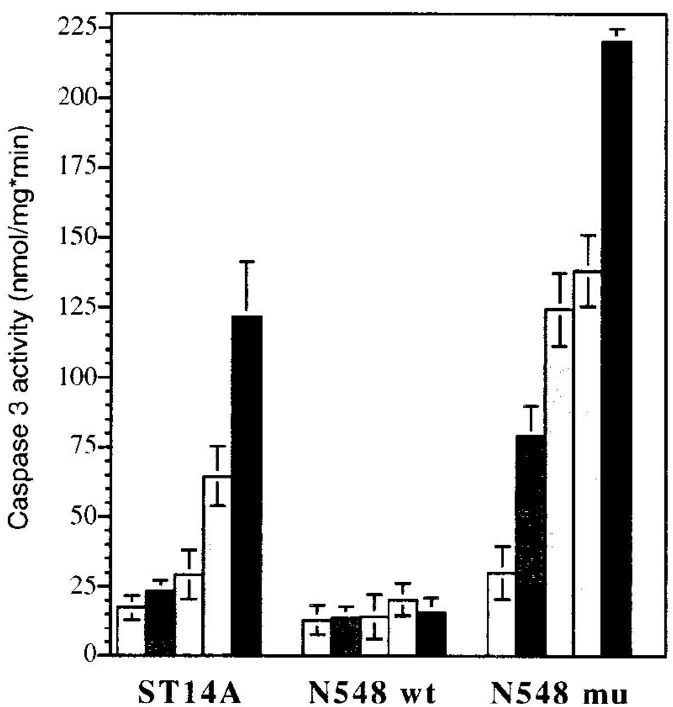
1

Wild-Type Huntingtin Protects from Apoptosis Upstream of Caspase-3

J. Neurosci 2000

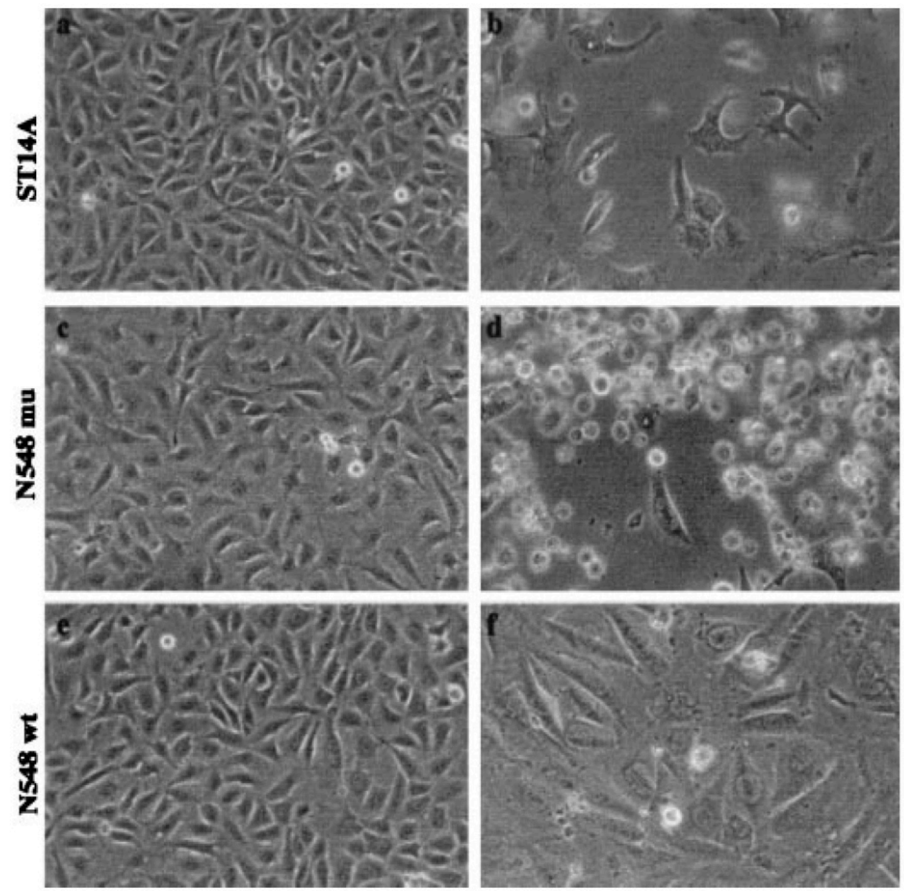
Dorotea Rigamonti,[1] Johannes H. Bauer,[2] Claudio De-Fraja,[1] Luciano Conti,[1] Simonetta Sipione,[1] Clara Sciorati,[3] Emilio Clementi,[3,4] Abigail Hackam,[5] Michael R. Hayden,[5] Yong Li,[2] Jillian K. Cooper,[6] Christopher A. Ross,[6] Stefano Govoni,[7] Claudius Vincenz,[2] and Elena Cattaneo[1]

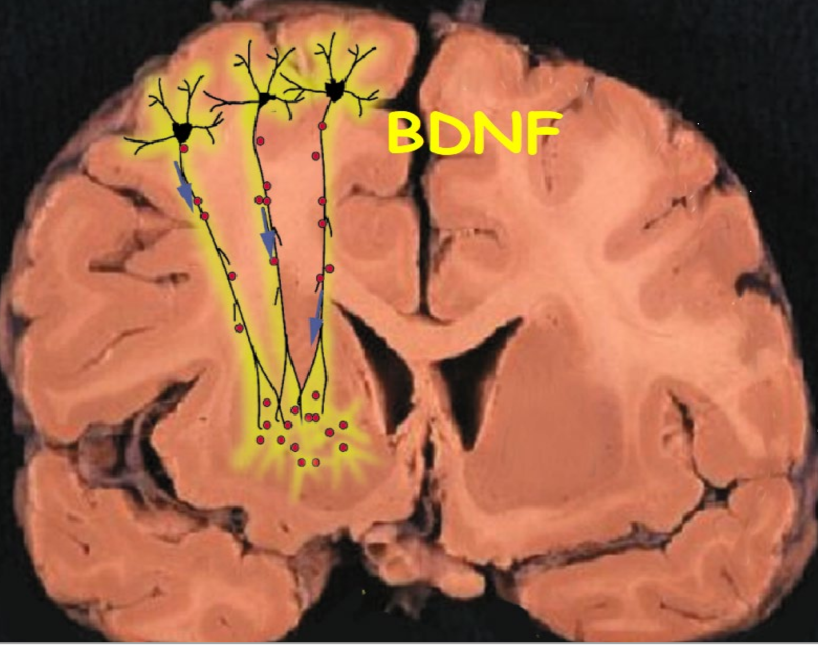
ST14A cells



33°C in serum

39°C in SFM





Loss of Huntingtin-Mediated BDNF Gene Transcription in Huntington's Disease

Chiara Zuccato,[1,2] Andrea Ciammola,[1,2,3] Dorotea Rigamonti,[1,2] Blair R. Leavitt,[4] Donato Goffredo,[1,2] Luciano Conti,[1,2] Marcy E. MacDonald,[5] Robert M. Friedlander,[6] Vincenzo Silani,[2,3] Michael R. Hayden,[4] Tõnis Timmusk,[7] Simonetta Sipione,[1,2] Elena Cattaneo[1,2]

Science 2001

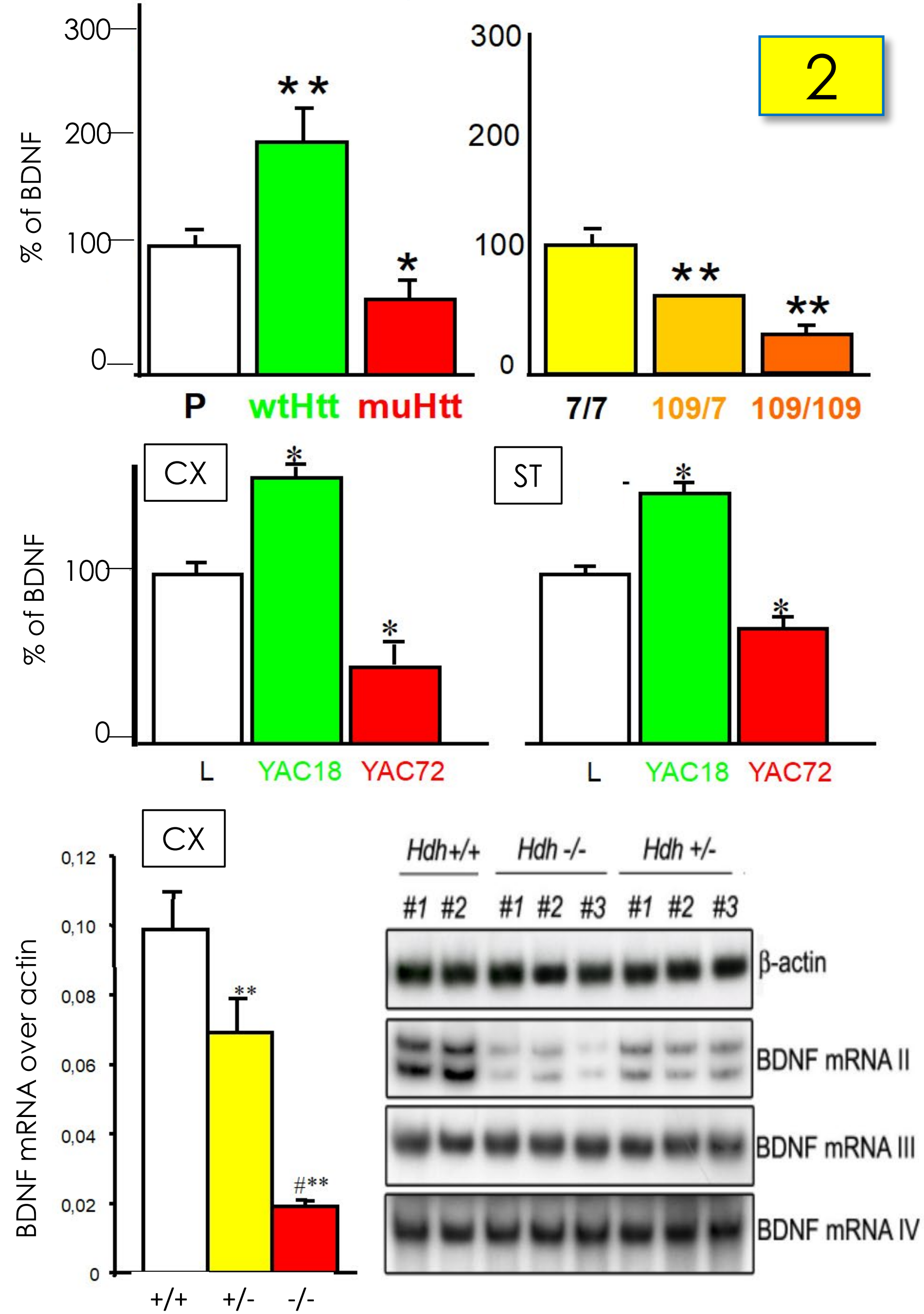
Huntingtin is a 350-kilodalton protein of unknown function that is mutated in Huntington's disease (HD), a neurodegenerative disorder. The mutant protein is presumed to acquire a toxic gain of function that is detrimental to striatal neurons in the brain. However, loss of a beneficial activity of wild-type huntingtin may also cause the death of striatal neurons. Here we demonstrate that wild-type huntingtin up-regulates transcription of brain-derived neurotrophic factor (BDNF), a pro-survival factor produced by cortical neurons that is necessary for survival of striatal neurons in the brain. We show that this beneficial activity of huntingtin is lost when the protein becomes mutated, resulting in decreased production of cortical BDNF. This leads to insufficient neurotrophic support for striatal neurons, which then die. Restoring wild-type huntingtin activity and increasing BDNF production may be therapeutic approaches for treating HD.

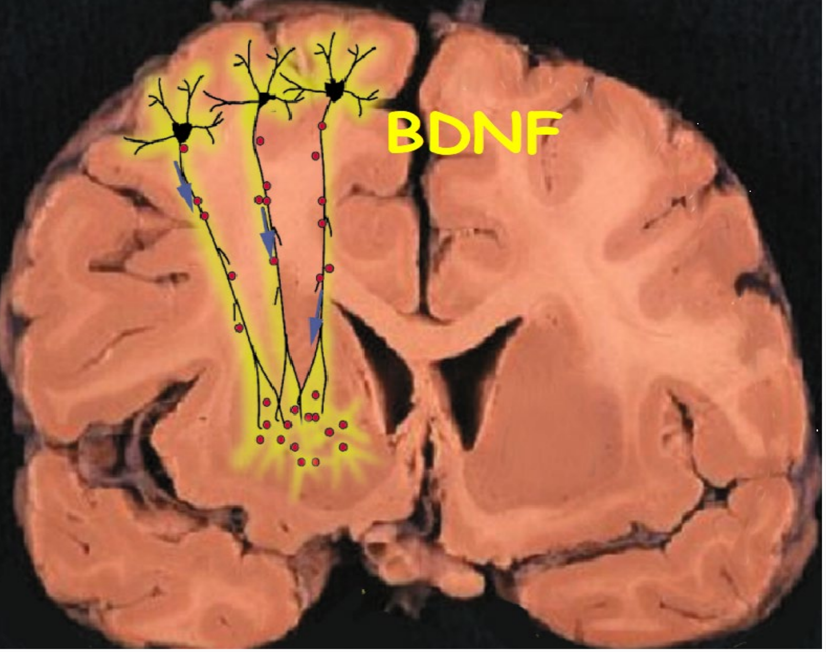
CELLS

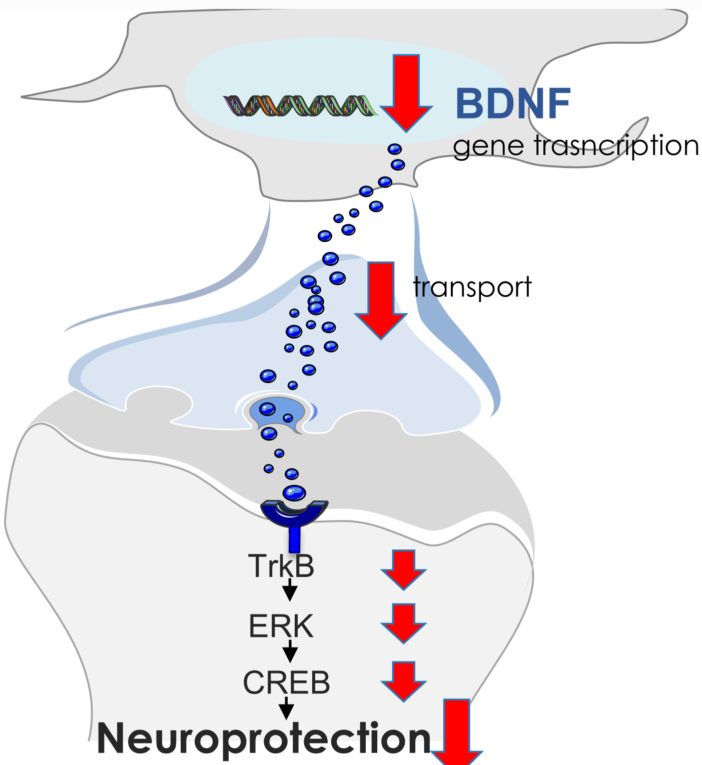
HD MICE

Cond KO MICE

wtHTT (but not mutant) stimulates BDNF production







BDNF production and delivery is dependent on wtHTT and is reduced in HD

HD cell lines
> 10 manuscripts show reduction in BDNF

HD mouse models
> 30 manuscripts show reduction in BDNF level in brain in
5 transgenic models
(R6/2; R6/1; YAC72, BACHD;N171-82Q)
3 knock-in models
(Hdh$^{111/111}$; Hdh$^{150/150}$; ZQ175)

HD human brain
3 papers show decreased BDNF levels

Human HD neurons from pluripotent stem cells
New unpublished data

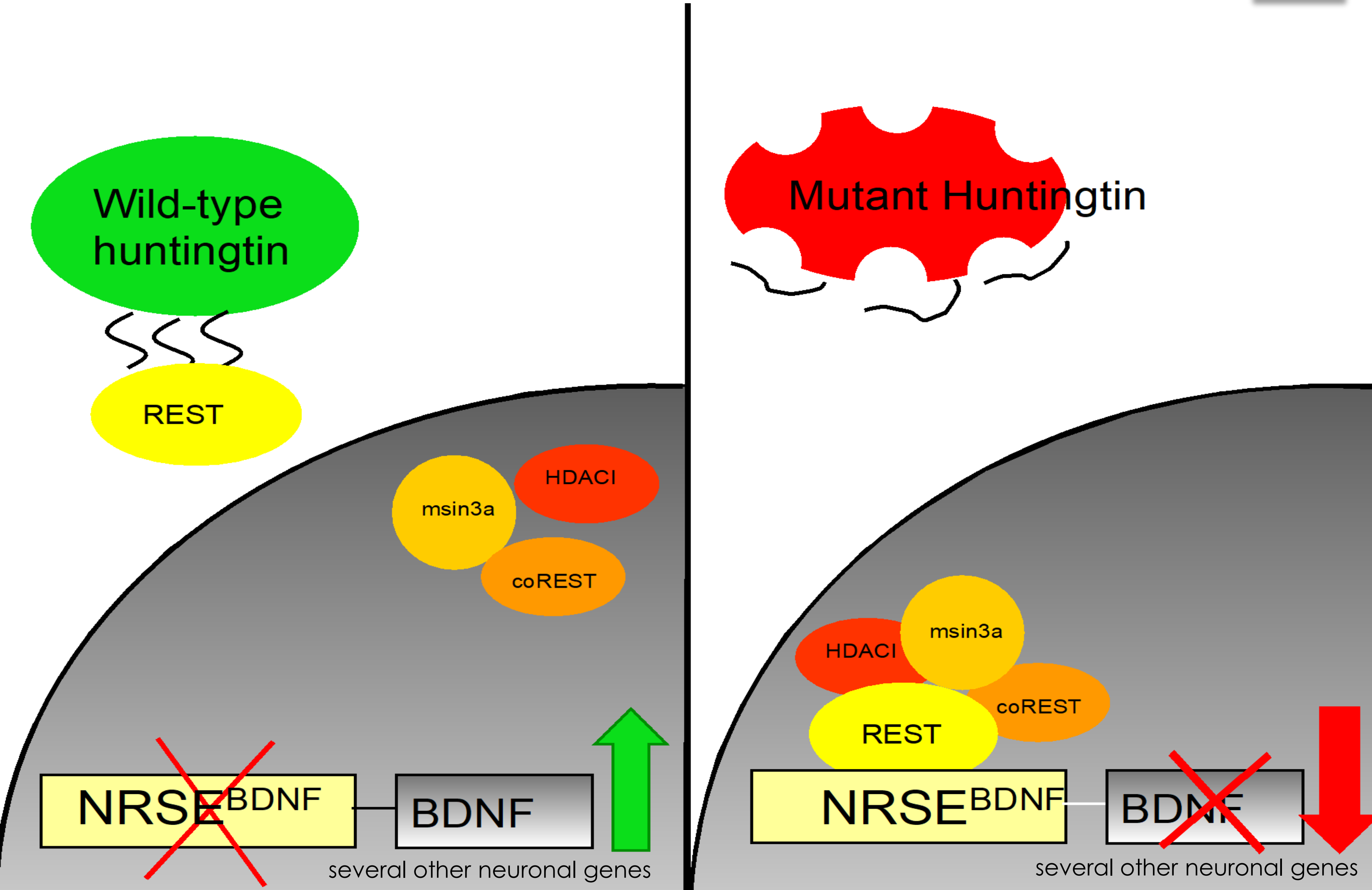
There is a BDNF "pathology" in HD which is due to **loss of wtHTT function**

2

Emx1-BDNF^{KO}	HD-like behavioral phenotype Gene expression changes similar to the ones in human HD caudate	Baquet, J. Neurosci, 2004 Strand, J. Neurosci, 2007
BDNF$^{+/-}$ R6/1	Earlier onset, worsening of the behavioral, motor phenotype Loss of striatal enkephalin-positive neurons	Canals, J. Neurosci, 2004
CamKIIα BDNF Tg;R6/1	Improvement of behavioral and motor phenotype Improvement of neuropathology and BDNF-mediated signalling	Gharami, *J. Neurochem, 2008*
CamKIIα BDNF Tg;YAC128	Less atrophy of striatal neurons Improvements of motor dysfunction	Xie, J. Neurosci, 2010

There is a BDNF/NRSE "pathology" in HD which is due to **loss of wtHTT function**

2



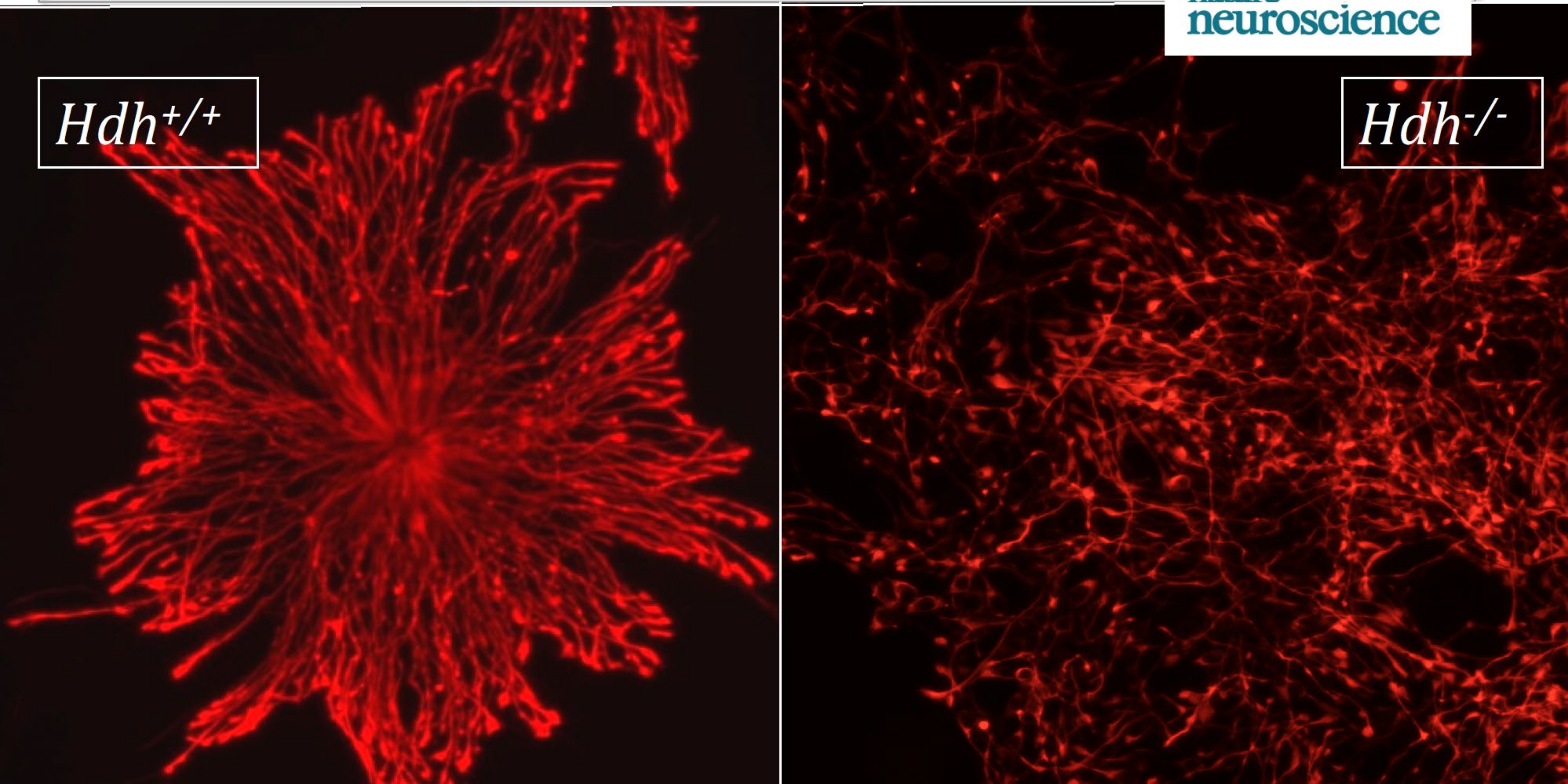
wtHTT (but not mutant) inhibits ADAM10

3

An evolutionary recent neuroepithelial cell adhesion function of huntingtin implicates ADAM10-Ncadherin

Valentina Lo Sardo, Chiara Zuccato, Germano Gaudenzi, Barbara Vitali, Catarina Ramos, Marzia Tartari, Michael A Myre, James A Walker, Anna Pistocchi, Luciano Conti, Marta Valenza, Binia Drung, Boris Schmidt, James Gusella, Scott Zeitlin, Franco Cotelli & Elena Cattaneo

nature neuroscience 2012



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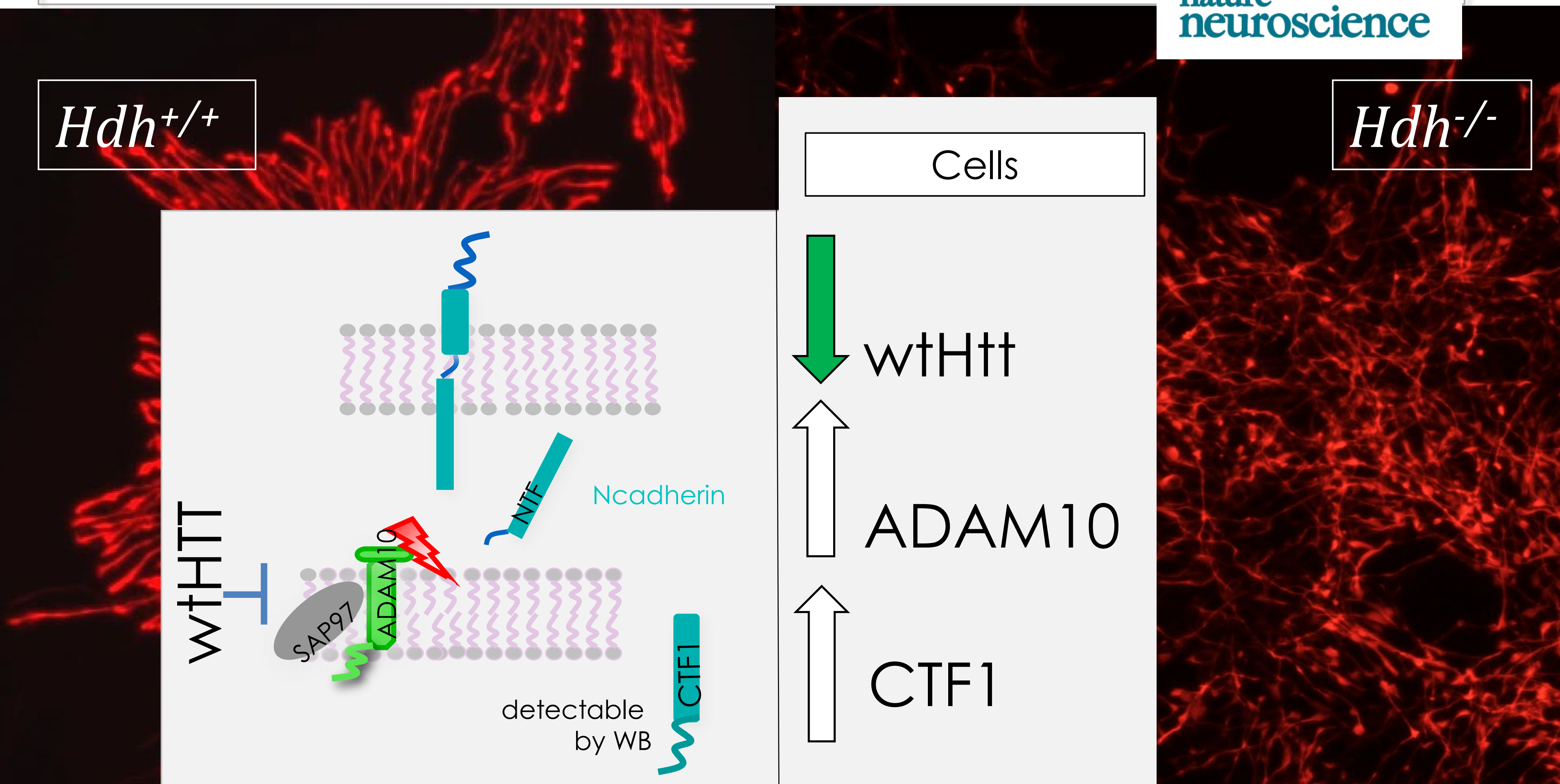

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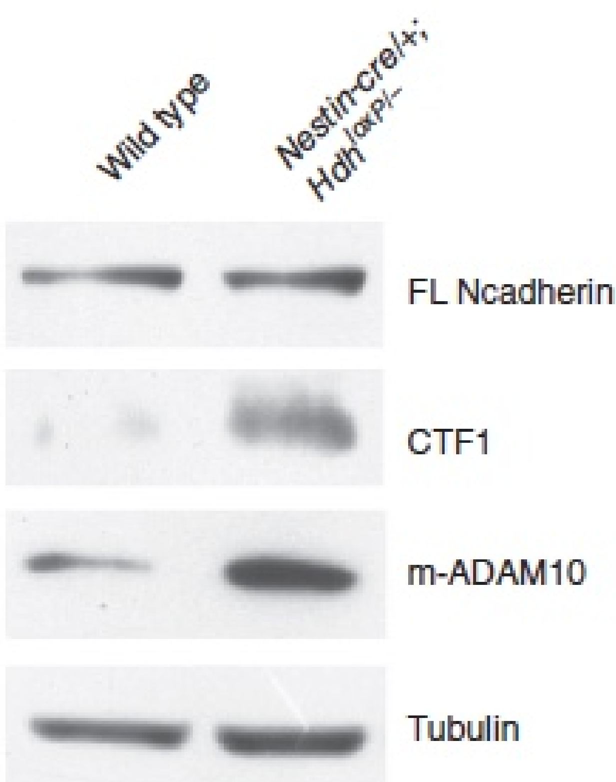
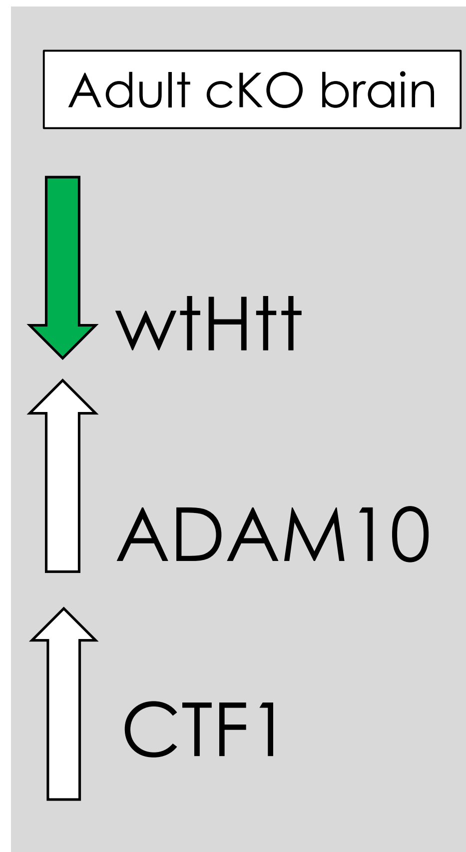
nature neuroscience 2012





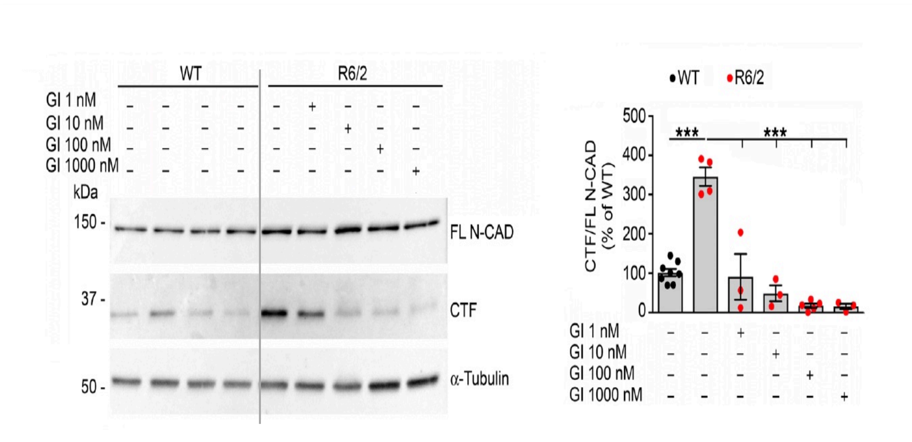
wtHTT (but not mutant) inhibits ADAM10

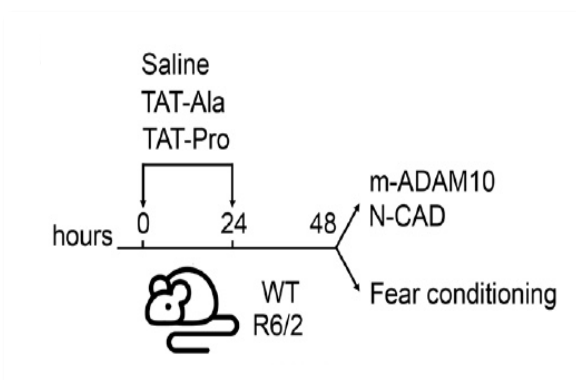
3

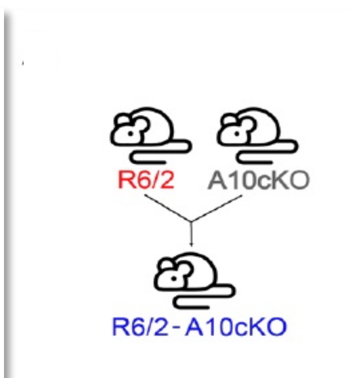
Inhibiting pathologically active ADAM10 rescues synaptic and cognitive decline in Huntington's disease

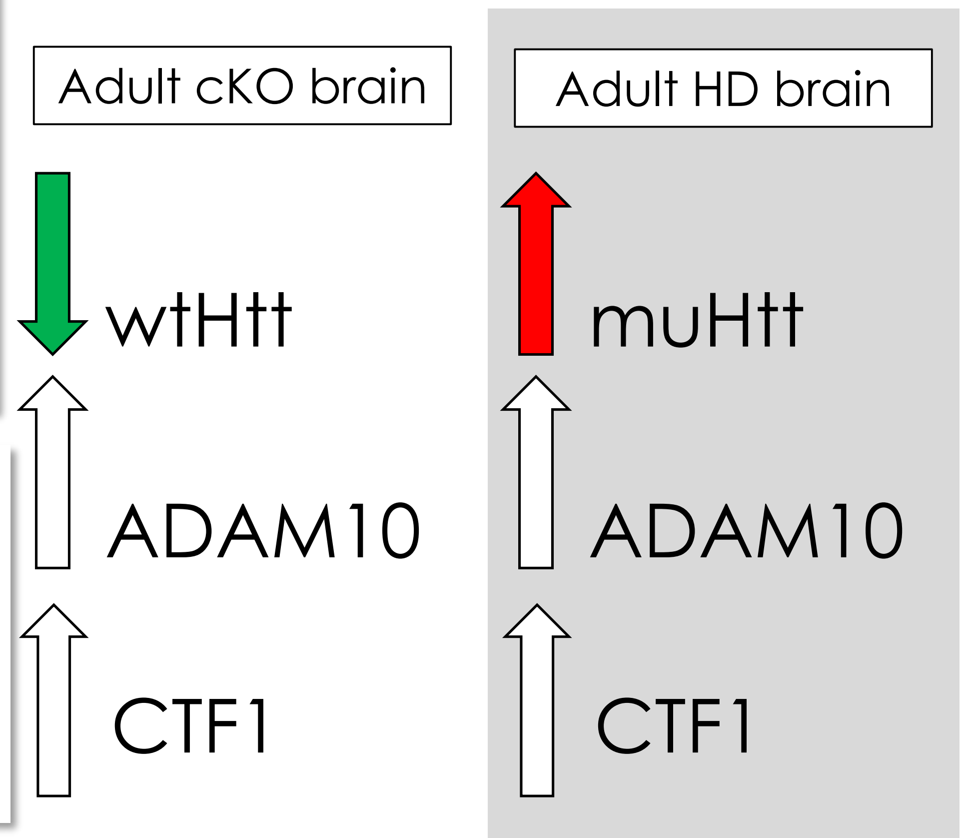
Elena Vezzoli,[1,2] Ilaria Caron,[1,2] Francesca Talpo,[3] Dario Besusso,[1,2] Paola Conforti,[1,2] Elisa Battaglia,[1,2] Elisa Sogne,[4] Andrea Falqui,[4] Lara Petricca,[5] Margherita Verani,[5] Paola Martufi,[5] Andrea Caricasole,[5] Alberto Bresciani,[5] Ottavia Cecchetti,[5] Pia Rivetti di Val Cervo,[1,2] Giulio Sancini,[6] Olaf Riess,[7] Hoa Nguyen,[7] Lisa Seipold,[8] Paul Saftig,[8] Gerardo Biella,[3] Elena Cattaneo,[1,2] and Chiara Zuccato[1,2]

Journal Clinical Investigation 2019









Conclusions

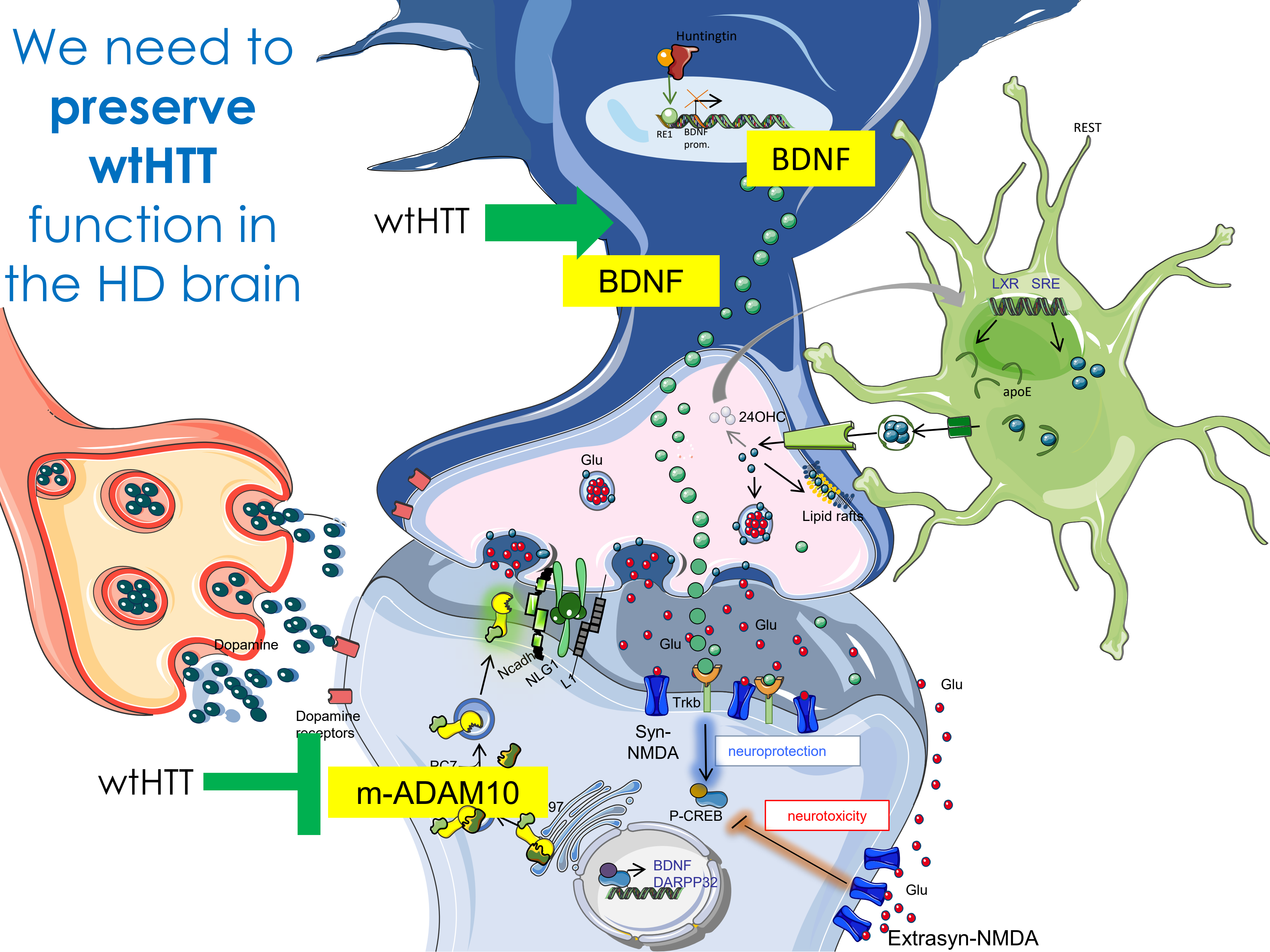
- muHTT toxicity causes nuclear, cytoplasmic and mitochondrial pathology; it affects **neurons** and **astrocytes;** it acts in a cell autonomous and **non-cell** autonomous manner; **toxicity can be reversed** by turning off muHTT expression; in a disease-modifying therapy **both striatum and cortex** should be targeted.

- for a **billion years** nature **has not** eliminated huntingtin but implemented its functions by lengthening its CAG

- HTT exerts different functions in the developing and adult brain

- In the adult brain wtHTT continues to be necessary and is **neuroprotective** while muHTT is unable to support, for example, BDNF production.

- Some phenotypes **in HD are due to loss of wtHTT function**,



We need to
preserve
wtHTT
function in
the HD brain
Huntingtin
RE1
BDNF prom.
BDNF
wtHTT
BDNF
REST
LXR SRE
apoE
24OHC
Glu
Lipid rafts
Dopamine
Dopamine receptors
Ncadh
NLG1
L1
Glu
Glu
Trkb
Glu
Syn-NMDA
neuroprotection
wtHTT
m-ADAM10
P-CREB
neurotoxicity
BDNF
DARPP32
Glu
Extrasyn-NMDA

Our lab in Milano


Chiara Zuccato
Associate Professor


Dario Besusso
Postdoctoral Fellow


Vittoria Bocchi
Postdoctoral Fellow


Giorgia Ferlazzo
Postdoctoral Fellow


Maura Galimberti
Postdoctoral Fellow

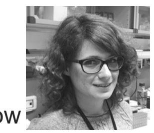

Raffaele Iennaco
Postdoctoral Fellow


Pia Rivetti
Postdoctoral Fellow


Camilla Trovesi
Postdoctoral Fellow


Marta Valenza
Researcher

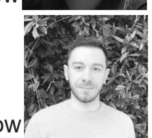

Elena Vezzoli
Postdoctoral Fellow

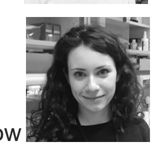

Martina Zobel
Postdoctoral Fellow


Paola Conforti
Specialized technologist


Giulia Birolini
PhD Student


Silvia Brocchetti
PhD Student


Manuel Cernigoj
PhD Student


Ilaria Campus
Junior Fellow


Andrea Scolz
Junior Fellow


Andrea Cossu
Junior Fellow

MANAGEMENT & PUBLIC OUTREACH


Gianni Munizza
Project Manager


Federico Tili
Lab Manager


Tiziana Turrisi
Grant & Pers. Manager

STUDENTS:
Tiziana Lischetti
Ayat Ahmed
Claudio Cappadona
Arianna Ferrari
Ivan Lombardi
Manuela Magni
Elena Melacini
Marta Vittani