Direct neural conversion:

New possibilities to model late onset neurodegenerative diseases

Karolina Pircs

HCEMM-SU Neurobiology

and Neurodegenerative diseases

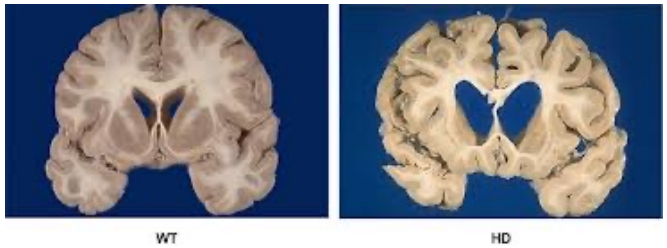
Biological therapies and vaccines: safety issues, physiological and immunological mechanisms

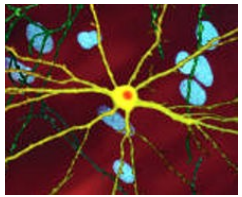
2021.06.15.

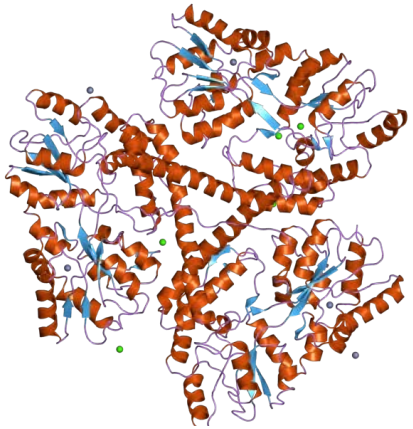
250 years of EXCELLENCE in medical education, research & innovation and healthcare

Huntington's Disease

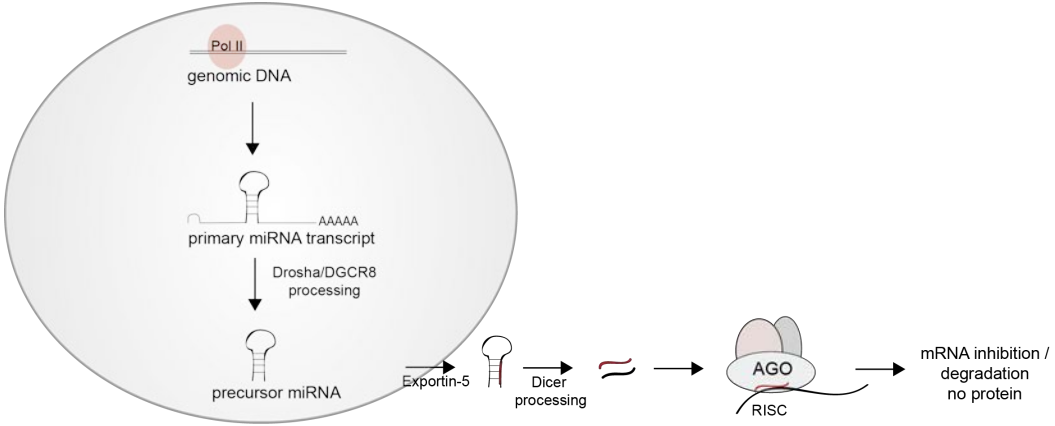
- Slowly progressive, hereditary brain disease that is characterized by motor dysfunction and cognitive decline
- Autosomal dominant disorder
- CAG repeats in the N-terminus of Huntingtin (HTT) gene resulting in protein aggregations of the mutant HTT







miRNAs



- small, single-stranded, non-coding RNAs
- 21-24 nt long
- bind to mRNAs and induce degradation or translational inhibition
- miRNAs are important post-transcriptional regulators
- involved in multiple cellular processes important for neuronal function
- linked to neurodegenerative diseases

miRNAs in HD

ELSEVIER

Neurobiology of Disease

www.elsevier.com/locate/ynbdi
Neurobiology of Disease 29 (2008) 438–445

A microRNA-based gene dysregulation pathway in Huntington's disease

Rory Johnson,[a,*] Chiara Zuccato,[b] Nikolai D. Belyaev,[c] Deborah J. Guest,[d] Elena Cattaneo,[b] and Noel J. Buckley[e]

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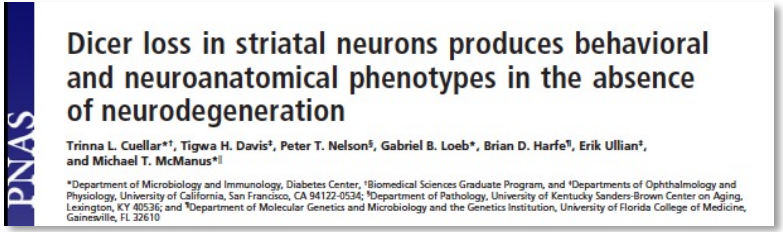

PNAS

Dicer loss in striatal neurons produces behavioral and neuroanatomical phenotypes in the absence of neurodegeneration

Trinna L. Cuellar*†, Tigwa H. Davis‡, Peter T. Nelson§, Gabriel B. Loeb*, Brian D. Harfe¶, Erik Ullian‡, and Michael T. McManus*‖

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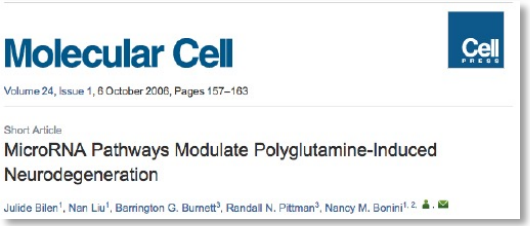


Molecular Cell

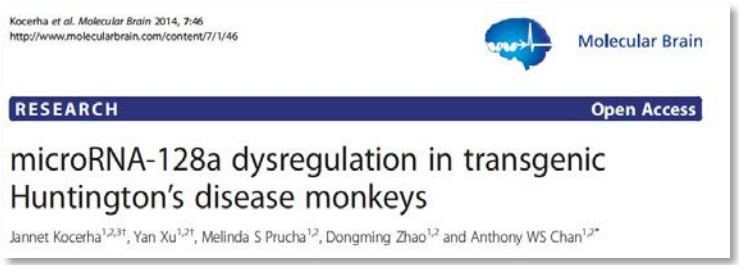
Cell PRESS

Volume 24, Issue 1, 6 October 2006, Pages 157–163

Short Article

MicroRNA Pathways Modulate Polyglutamine-Induced Neurodegeneration

Julide Bilen[1], Nan Liu[1], Barrington G. Burnett[3], Randall N. Pittman[3], Nancy M. Bonini[1, 2, *]



Kocerha et al. Molecular Brain 2014, 7:46
http://www.molecularbrain.com/content/7/1/46

Molecular Brain

RESEARCH Open Access

microRNA-128a dysregulation in transgenic Huntington's disease monkeys

Jannet Kocerha[1,2,3†], Yan Xu[1,2†], Melinda S Prucha[1,2], Dongming Zhao[1,2] and Anthony WS Chan[1,2*]

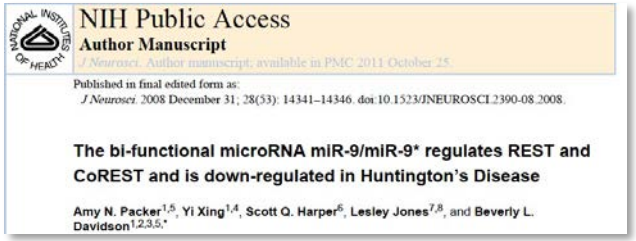
Hoss et al. BMC Medical Genomics (2015) 8:10
DOI 10.1186/s12920-015-0083-3

BMC Medical Genomics

RESEARCH ARTICLE Open Access

miR-10b-5p expression in Huntington's disease brain relates to age of onset and the extent of striatal involvement

Andrew G Hoss[1,2], Adam Labadorf[1,3], Jeanne C Latourelle[1], Vinay K Kartha[3], Tiffany C Hadzi[1], James F Gusella[4], Marcy E MacDonald[4], Jiang-Fan Chen[1], Schahram Akbarian[5], Zhiping Weng[6], Jean Paul Vonsattel[7] and Richard H Myers[1,8*]



NIH Public Access
Author Manuscript
J Neurosci. Author manuscript; available in PMC 2011 October 25.

Published in final edited form as:
J Neurosci. 2008 December 31; 28(53): 14341–14346. doi:10.1523/JNEUROSCI.2390-08.2008.

The bi-functional microRNA miR-9/miR-9* regulates REST and CoREST and is down-regulated in Huntington's Disease

Amy N. Packer[1,5], Yi Xing[1,4], Scott Q. Harper[6], Lesley Jones[7,8], and Beverly L. Davidson[1,2,3,5,*]

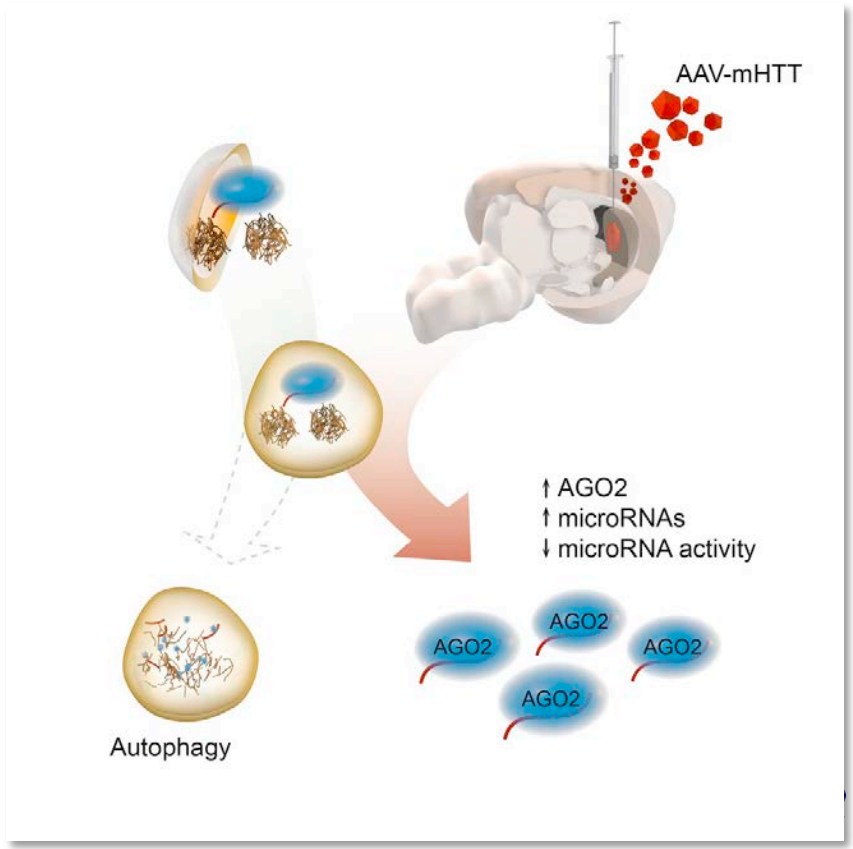
Cell Reports
Report

OPEN ACCESS
CellPress

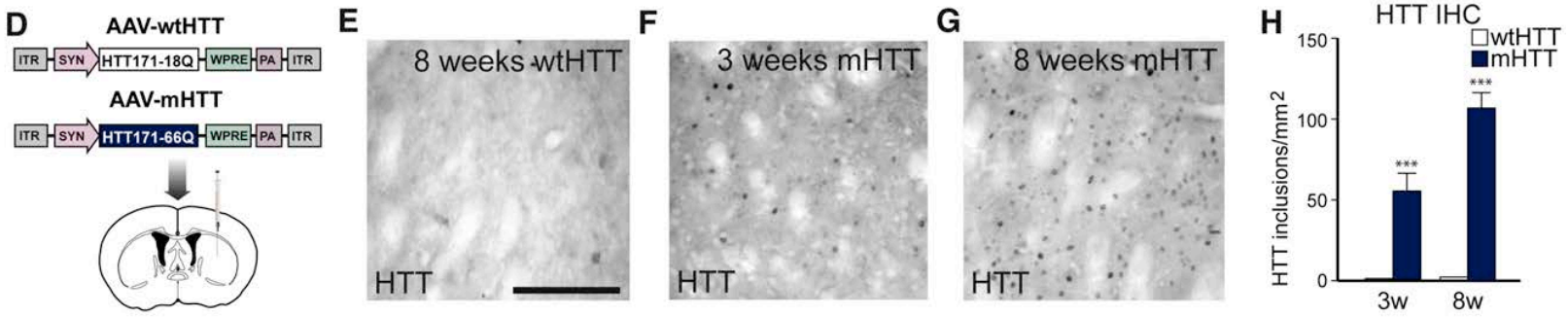
Huntingtin Aggregation Impairs Autophagy, Leading to Argonaute-2 Accumulation and Global MicroRNA Dysregulation

Karolina Pircs,[1] Rebecca Petri,[1] Sofia Madsen,[1] Per Ludvik Brattås,[1] Romina Vuono,[2] Daniella R. Ottosson,[3] Isabelle St-Amour,[4] Bob A. Hersbach,[1] Monika Matusiak-Brückner,[1] Sofia Hult Lundh,[5] Åsa Petersén,[5] Nicole Déglon,[6] Sébastien S. Hébert,[4] Malin Parmar,[3] Roger A. Barker,[2,3] and Johan Jakobsson[1,7,*]

[1]Laboratory of Molecular Neurogenetics, Department of Experimental Medical Science, Wallenberg Neuroscience Center and Lund Stem Cell Center, BMC A11, Lund University, 221 84 Lund, Sweden



Expression of mHTT in mouse striatum results in AGO2 accumulation



Pircs et al. Cell Reports, 2017

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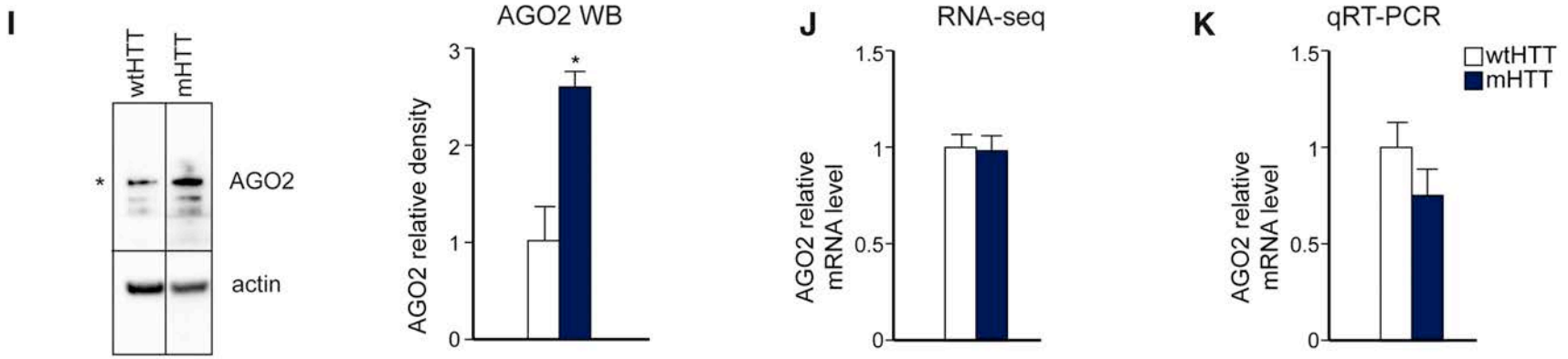

Pircs et al. Cell Reports, 2017

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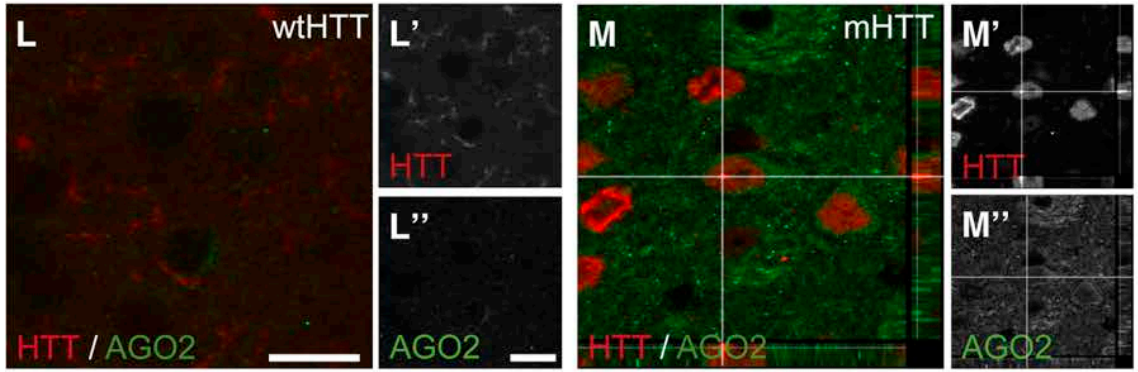
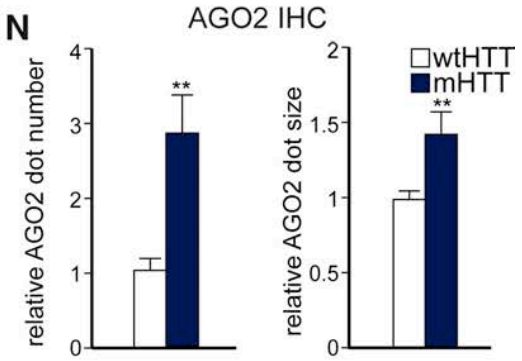
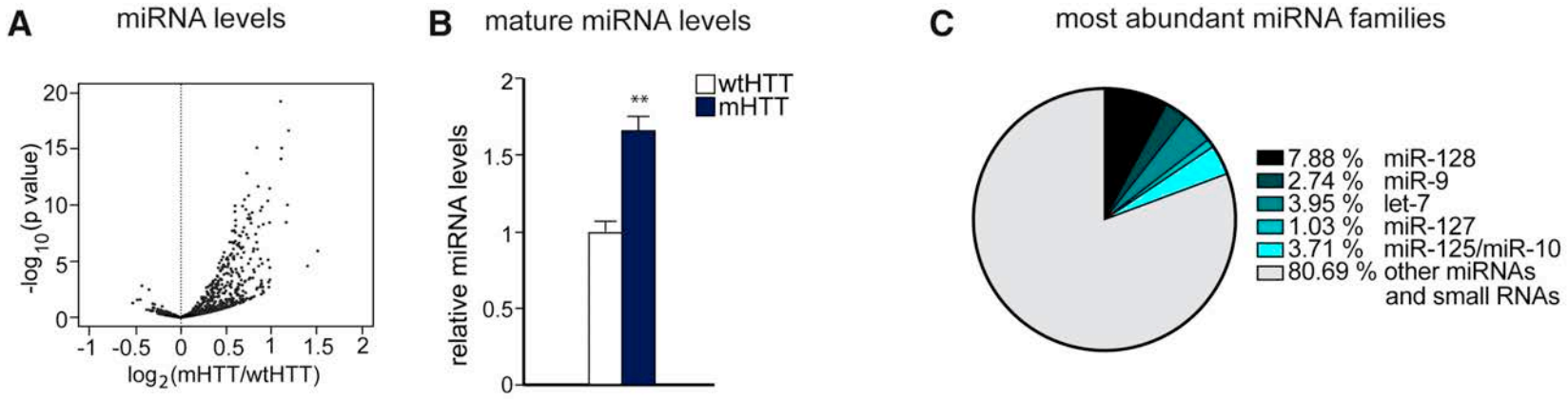




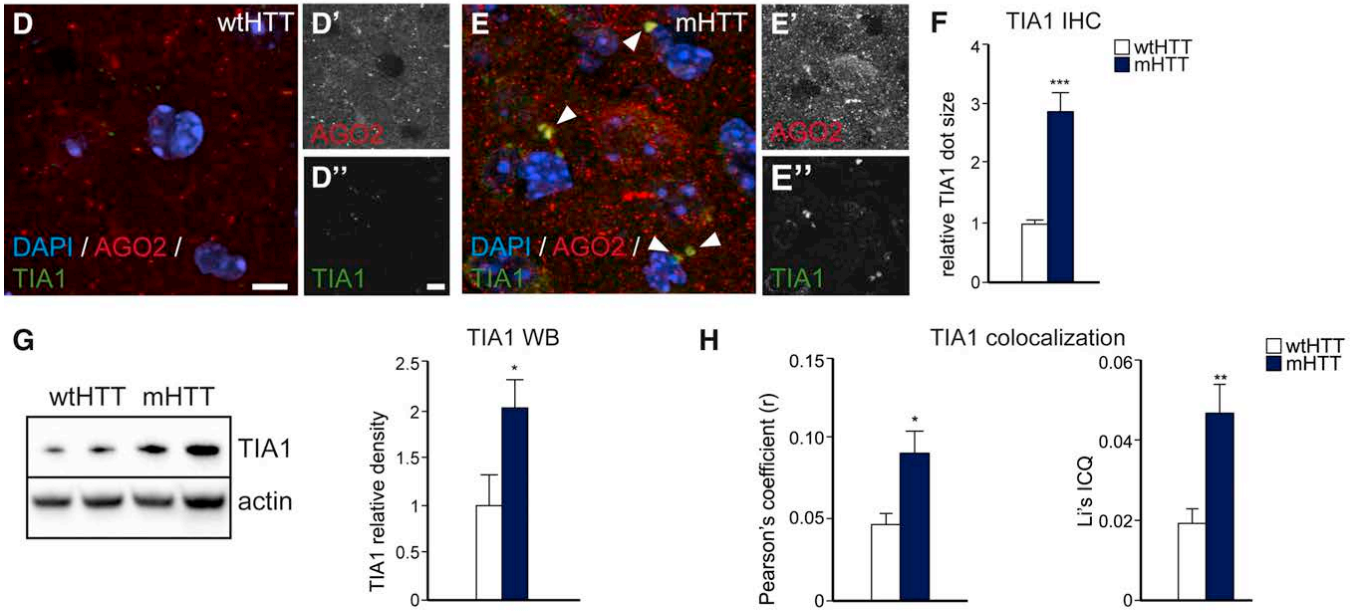
Pircs et al. Cell Reports, 2017

Accumulation of AGO2 results in more miRNAs… but these are trapped in stress granules



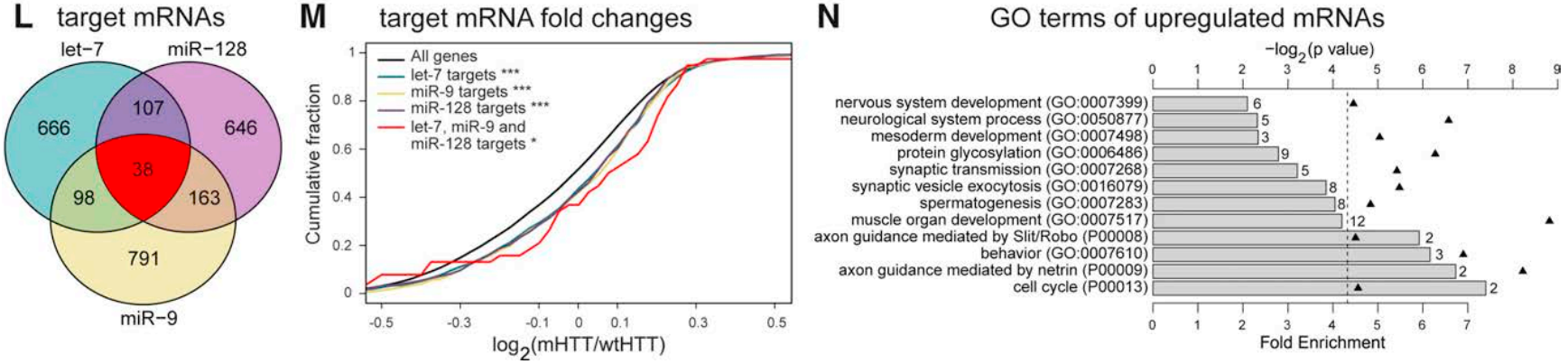
Pircs et al. Cell Reports, 2017

Accumulation of AGO2 results in more miRNAs… but these are trapped in stress granules



Pircs et al. Cell Reports, 2017

Results in a loss of miRNA-activity



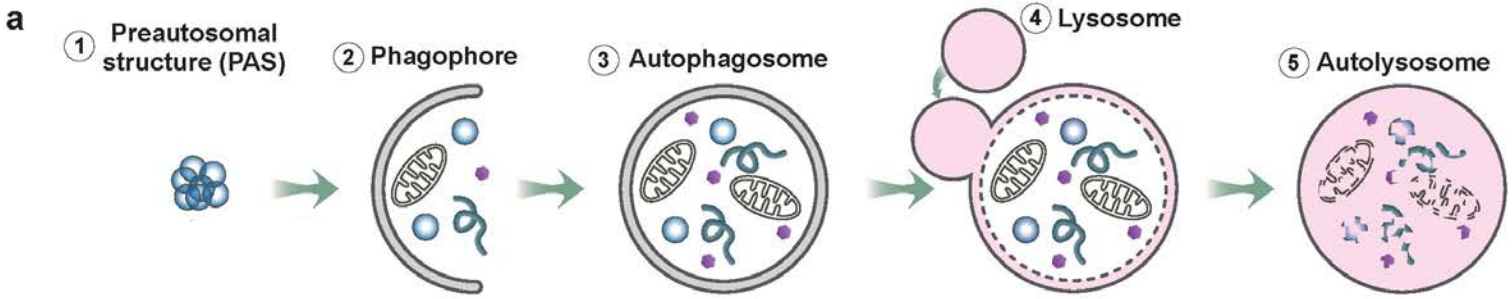
Pircs et al. Cell Reports, 2017

Conclusions

- These results suggest:
  - changes in miRNA levels are an early feature of HD
  - lies downstream of alterations in autophagy

Autophagy

- Autophagy is a highly conserved lysosomal degradation pathway
- Basal and selective autophagy have both been shown to be crucial for neuronal functions



Pircs et al. in revision, 2021,
BioRxiv preprint available at https://doi.org/10.1101/2021.03.01.433433

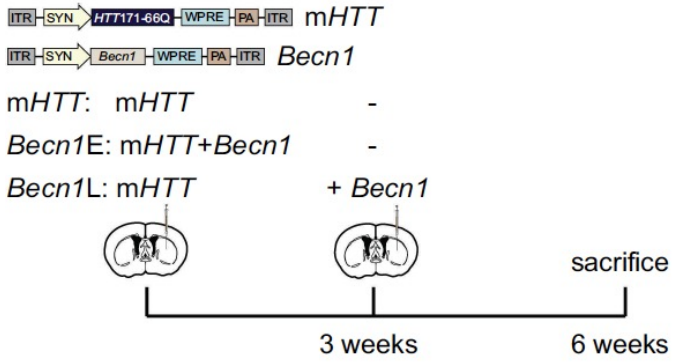
Autophagy

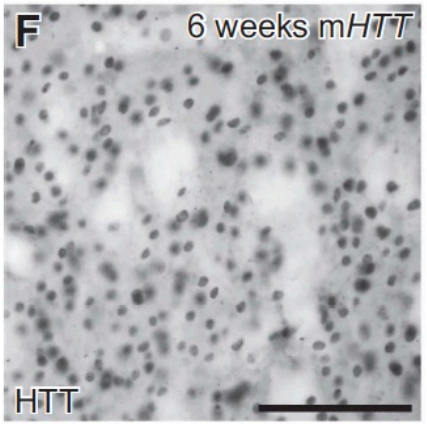
Autophagy

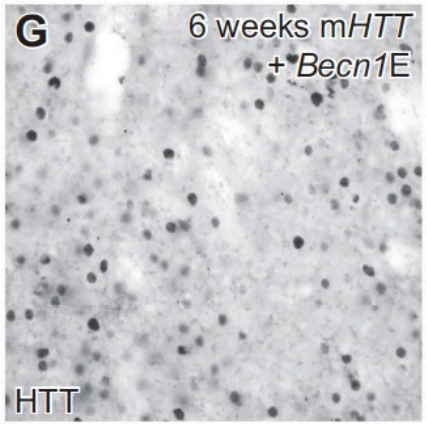
ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/kaup20

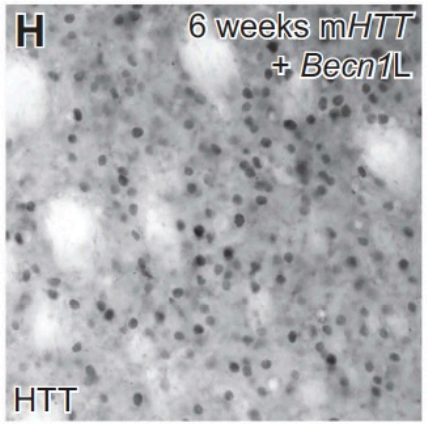
Impact of differential and time-dependent autophagy activation on therapeutic efficacy in a model of Huntington disease

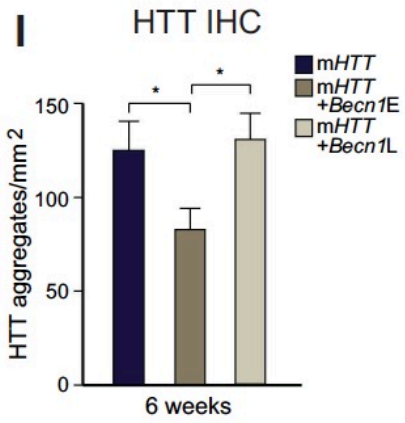
Per Ludvik Brattås , Bob A. Hersbach , Sofia Madsen , Rebecca Petri , Johan Jakobsson & Karolina Pircs











Conclusions

- These results provide further support for developing autophagy-activating therapeutic approaches for HD
- activation of autophagy will not only clear toxic protein aggregates but also
  - directly restore dysfunctional post-transcriptional gene regulation.

- But…. are these results relevant for Huntington´s disease?
- Is autophagy actually impaired in HD?

Experimental systems to study Huntington's Disease

How do we test a drug before use in humans?

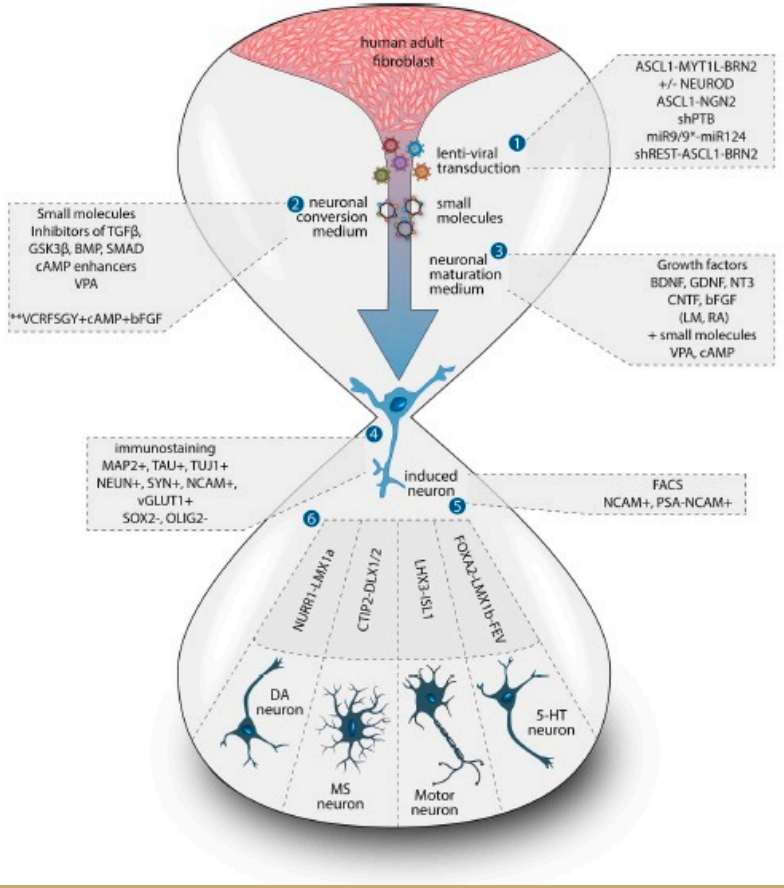
Can we predict success with todays models?

Development of a new Huntington's Disease model

- Human origin
- Patient derived and disease specific neurons
- Short CAG-repeat length
- Maintain age
- Study many patients

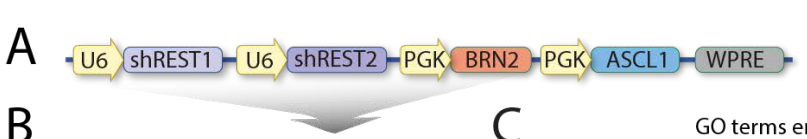
Patient-derived induced neurons (iNs)

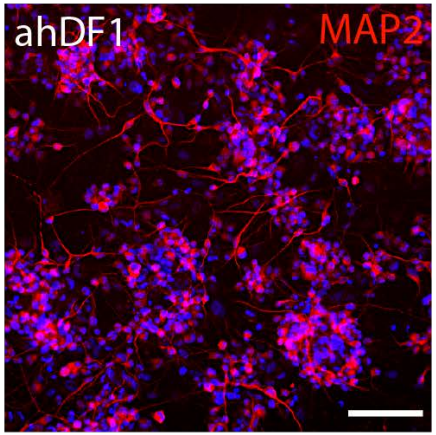
- Fibroblasts harvested from patients with HD can be reprogrammed to neurons
- Neurons contain the genetic and aging related signatures of the donor

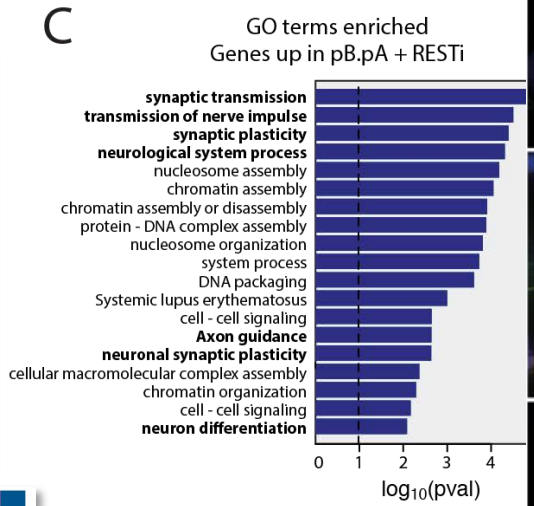


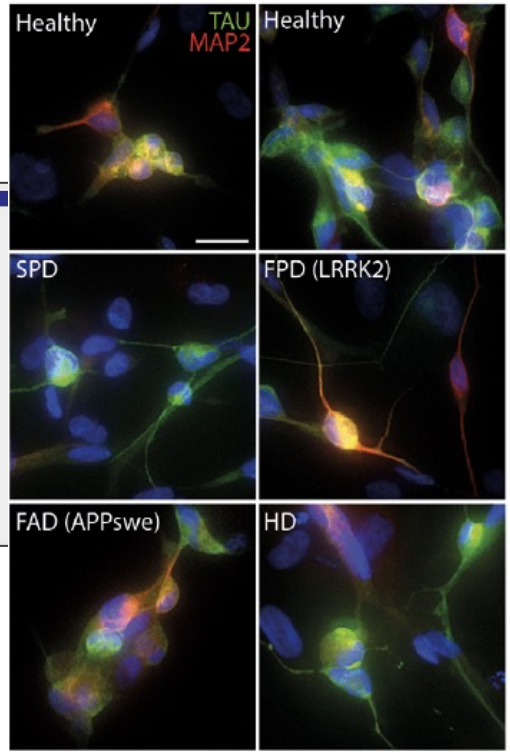
Drouin-Ouellet et al. Frontiers in Neuroscience, 2017

Highly efficient one-step generation of iNs from patients with HD, PD, AD using all-in-one novel vector system









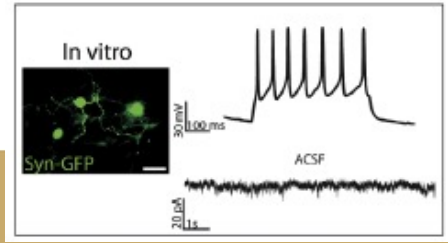
Published online: June 23, 2017

Research Article

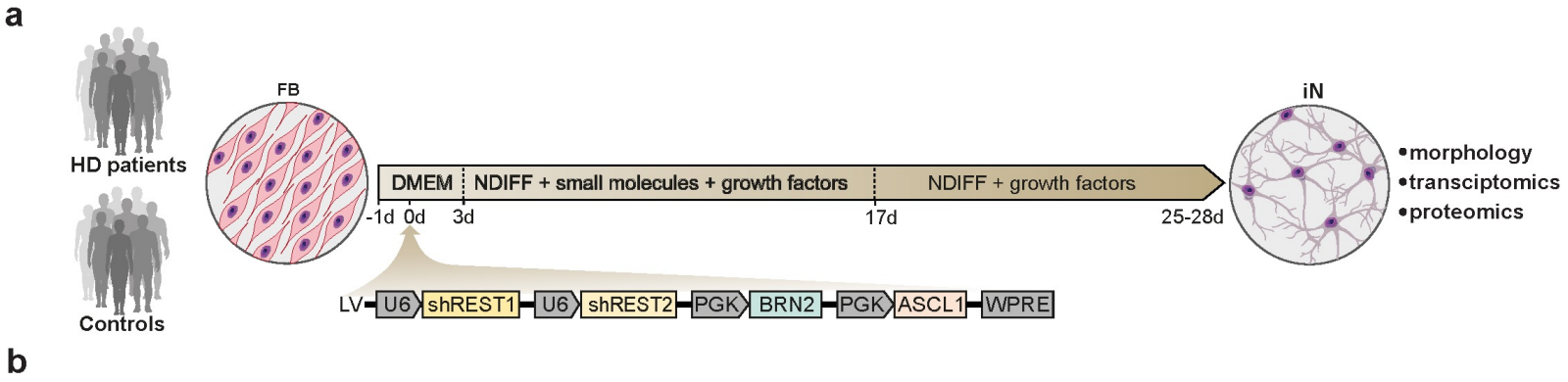
EMBO Molecular Medicine

REST suppression mediates neural conversion of adult human fibroblasts via microRNA-dependent and -independent pathways

Janelle Drouin-Ouellet[1,†], Shong Lau[1,†], Per Ludvik Brattås[1], Daniella Rylander Ottosson[1], Karolina Pircs[1], Daniela A Grassi[1], Lucy M Collins[2], Romina Vuono[2], Annika Andersson Sjöland[3], Gunilla Westergren-Thorsson[3], Caroline Graff[4,5], Lennart Minthon[6], Håkan Toresson[6], Roger A Barker[1,2], Johan Jakobsson[1] & Malin Parmar[1,*]



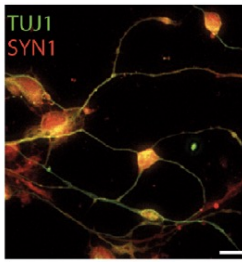
Highly efficient one-step generation of iNs from patients with HD using all-in-one novel vector system

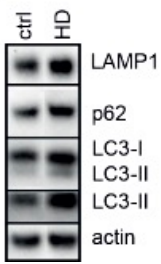


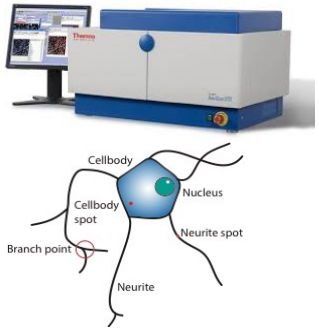
Pircs et al. in revision, 2021,
BioRxiv preprint available at https://doi.org/10.1101/2021.03.01.433433

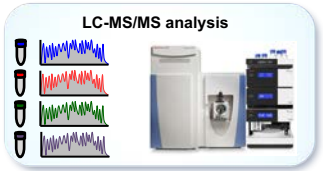
Highly efficient one-step generation of iNs from patients with HD using all-in-one novel vector system

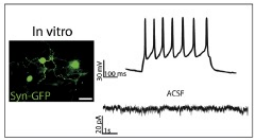
- From 50.000 fibroblast cells we get >10.000 neurons

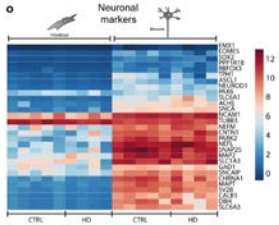


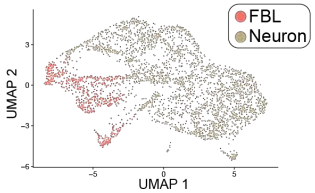












Drouin-Oullet et al., 2017,
Pircs et al. in revision, 2021,
BioRxiv preprint available at https://doi.org/10.1101/2021.03.01.433433

We have studied using iNs…

Line	Age	Sex	CAG repeats
C1	27	M	17
C2	30	M	19-24
C3	52	F	19-23
C4	54	F	15-20
C5	61	F	17
C6	61	M	17-23
C7	66	M	24
C8	67	F	n/a
C9	71	M	n/a
C10	75	F	18
HD1	28	M	15-39
HD2	31	M	20-45
HD3	33	F	17-58
HD4	38	F	17-52
HD5	43	M	17-42
HD6	43	M	19-44
HD7	47	M	40
HD8	49	F	18-47
HD9	53	M	19-42
HD10	59	M	16-39

Pircs et al. in revision, 2021,
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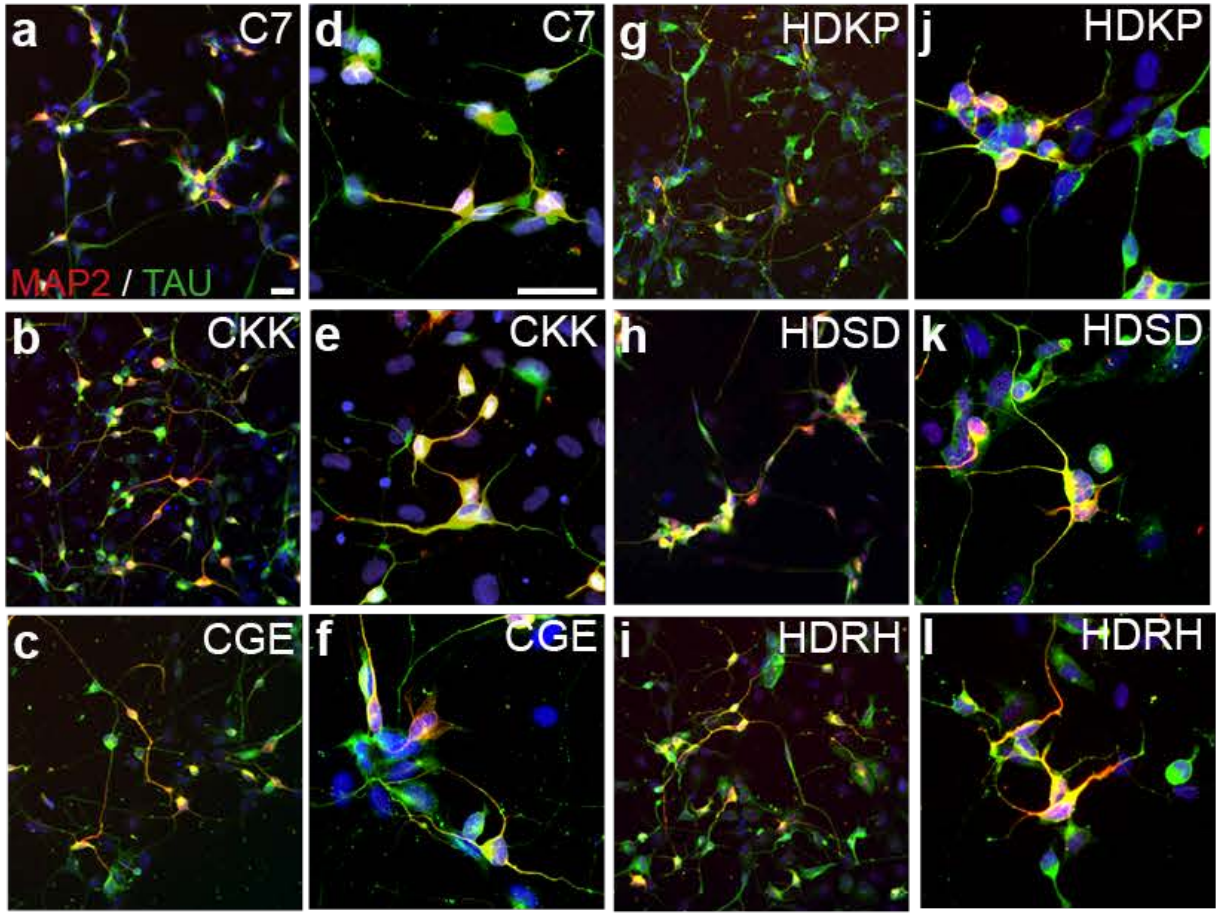

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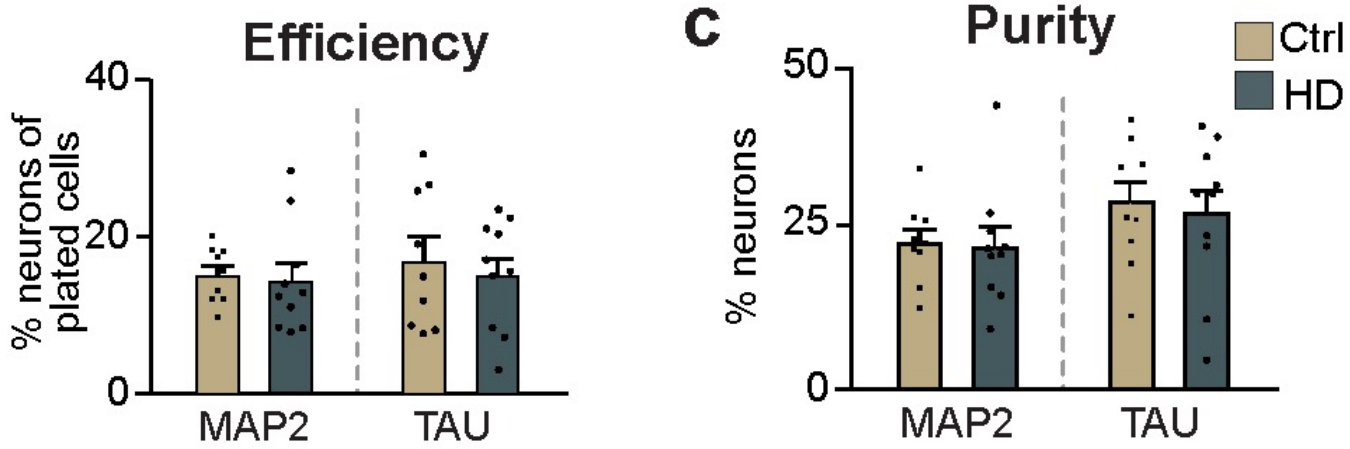
High-content automated microscopy (HCA)

- Morphologically characterize cell lines after 28 days of conversion
- Methods: ICC
  - neuronal markers (TAU, MAP2)
- Analysis: HCS
  - Target activation:
    → Measurement of fluorescent indicators on a cell-by-cell basis. We can define: conversion efficiency and neuronal purity
  - Neuronal profiling:
    → Quantification of morphological characteristics in neuronal cells

HD cell lines readily convert into iNs



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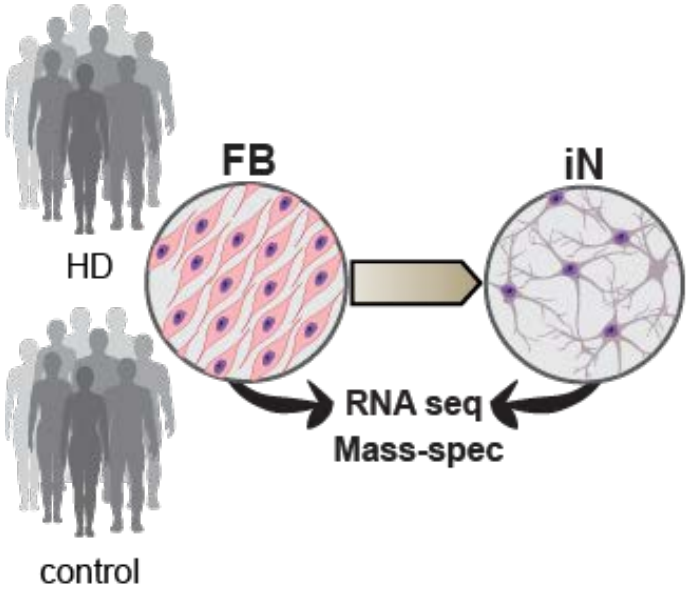
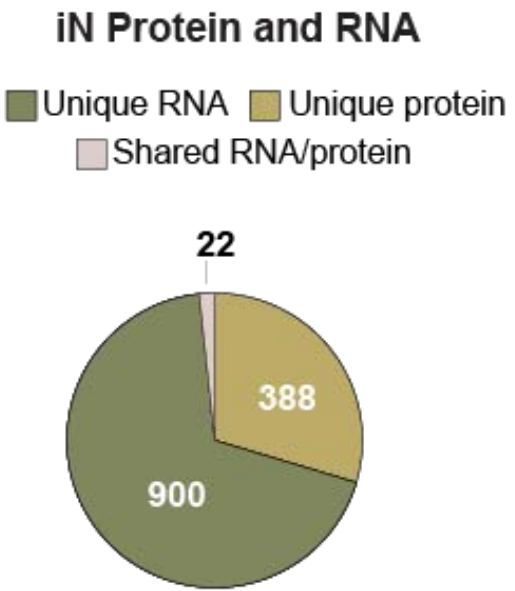
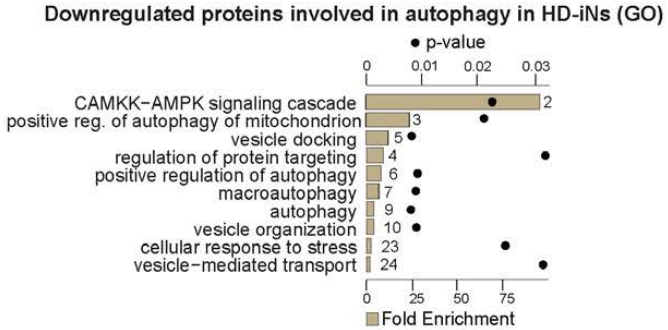
Transcriptome and proteome profiling of iNs

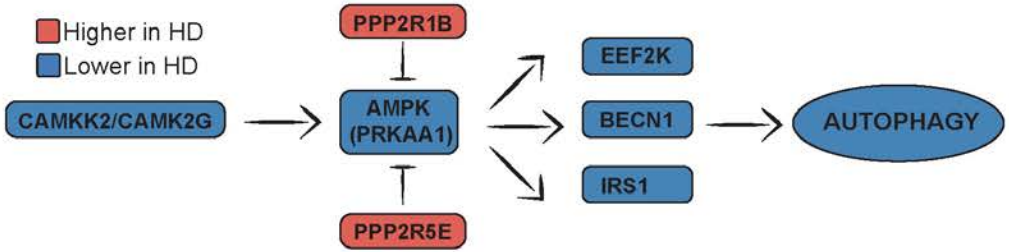




Pircs et al. in revision, 2021,
BioRxiv preprint available at https://doi.org/10.1101/2021.03.01.433433

HD-iNs display alterations in proteins linked to autophagy: cell type specific, only proteins!





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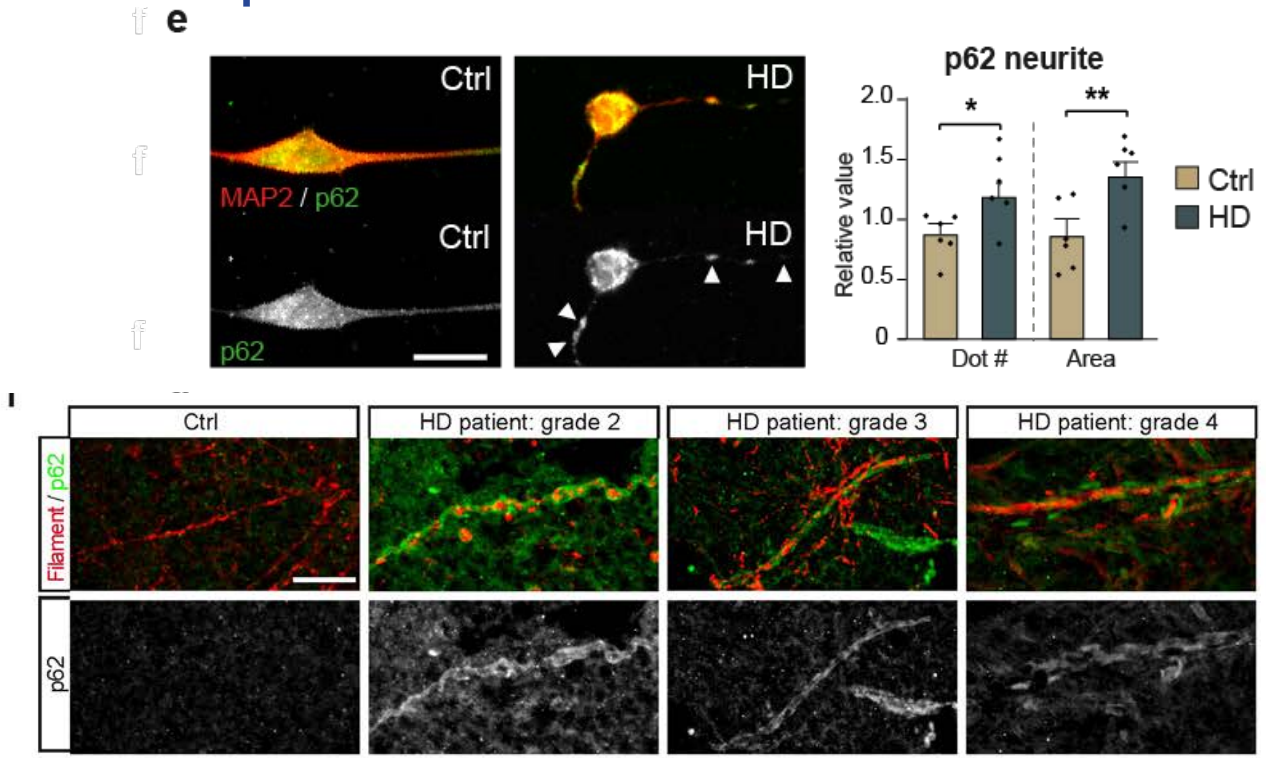
Subcellular alterations in autophagy in HD-iNs Verified in postmortem HD striatal tissue



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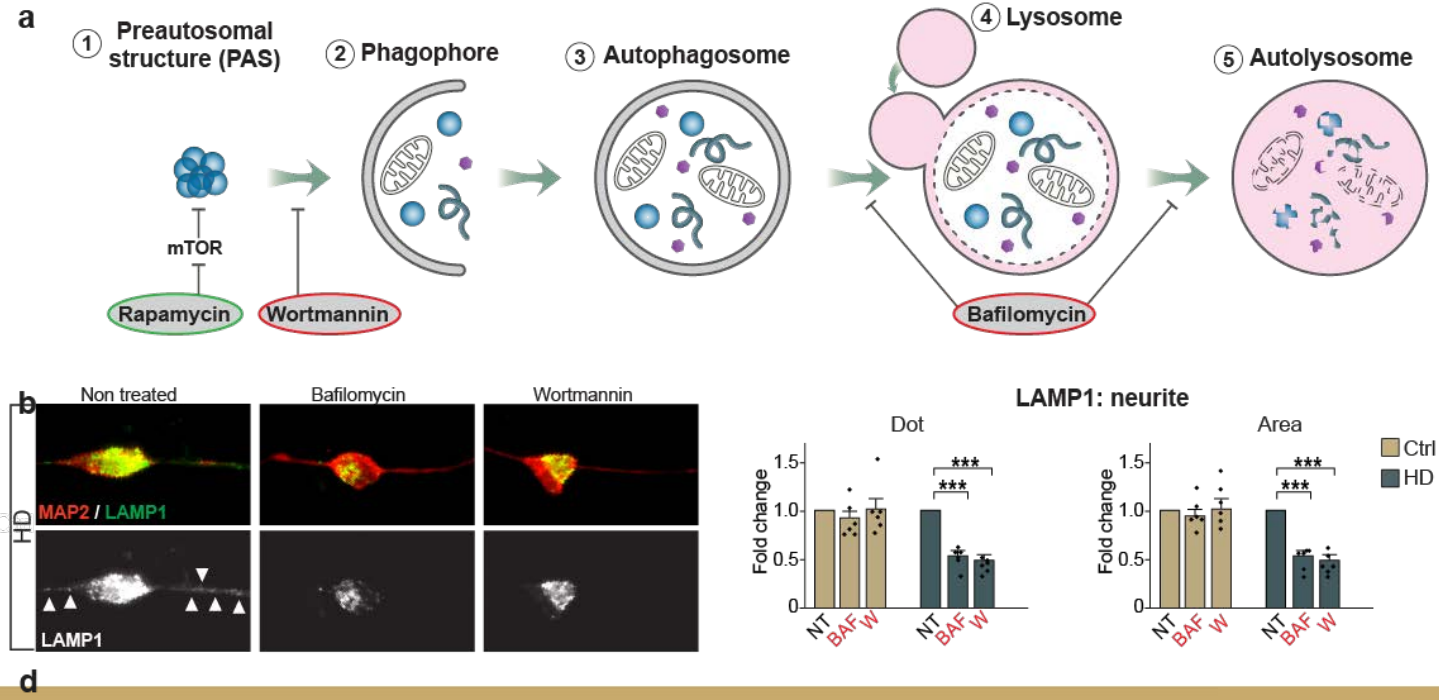
Impaired autophagic flux in HD-iN: probably due to retrograde transport failure



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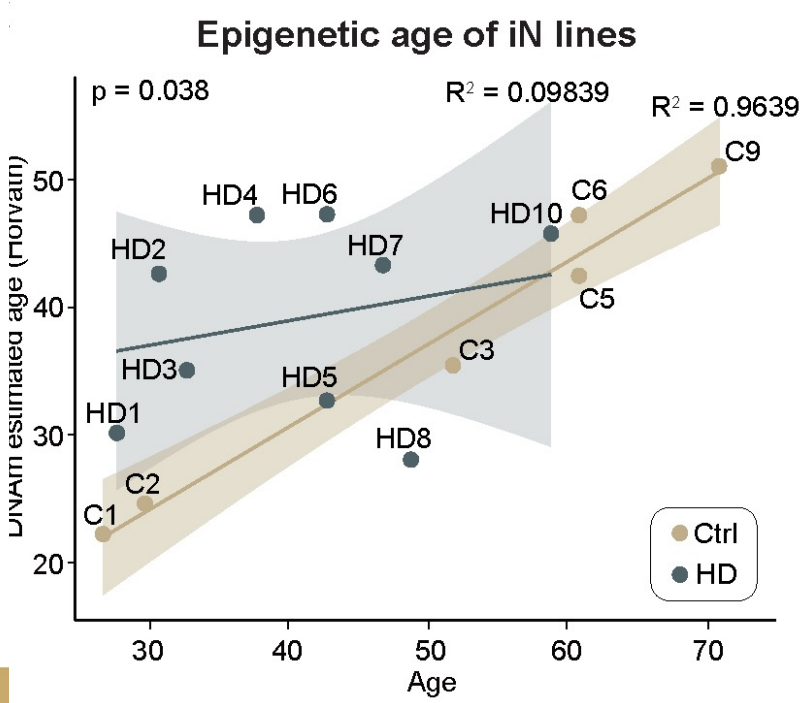
Investigate the cellular mechanisms underlying the autophagy impairments found in HD-iNs: 1. AGEING



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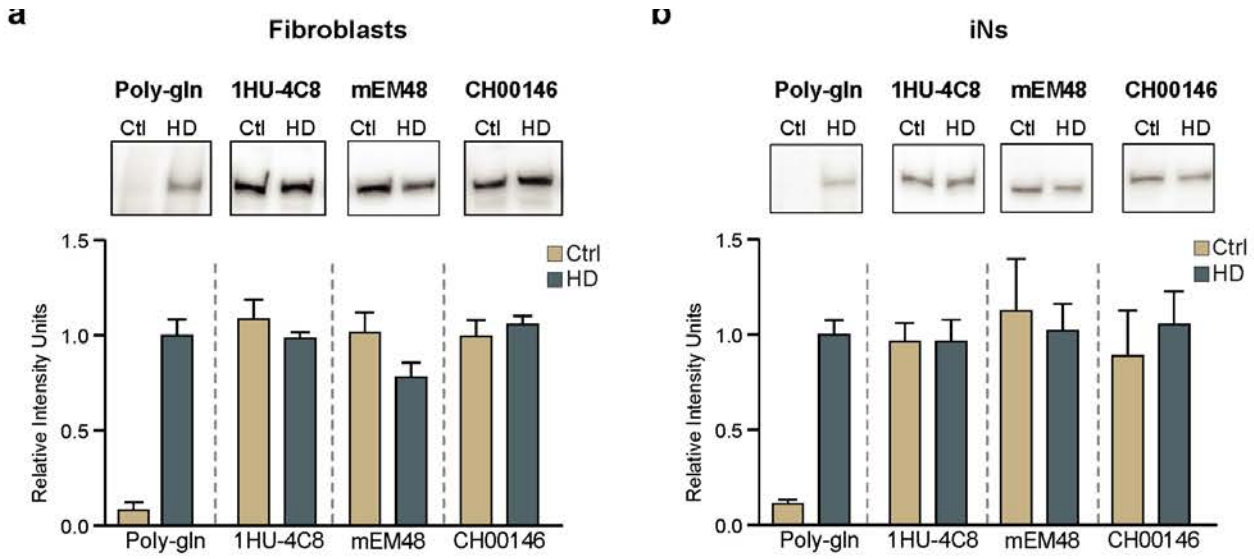
2. mHTT Aggregates
No mHTT aggregation in these cells



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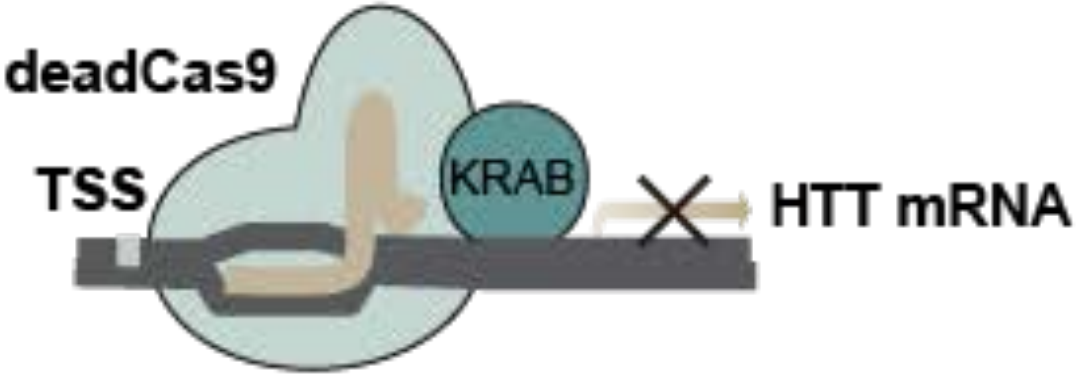
What is the direct role of HTT in the regulation of autophagy in iNs? CRISPRi



- dCas9 fused to a transcriptional repressor, KRAB
- Silencing gene expression by interfering with transcription

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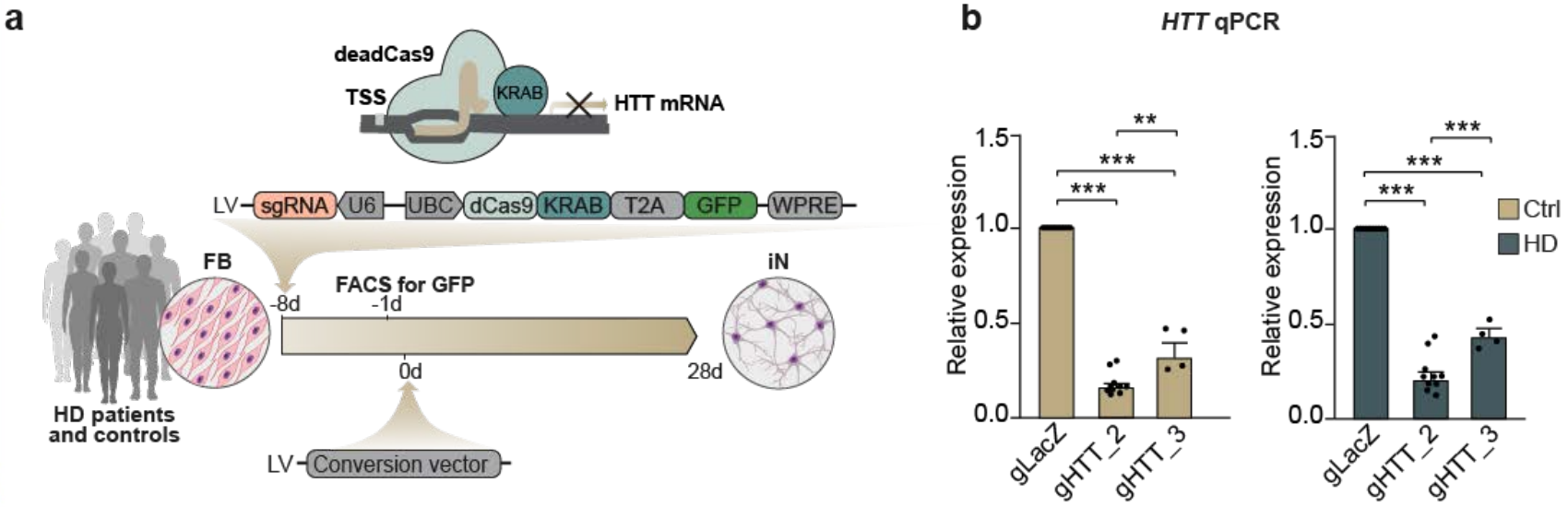
What is the direct role of HTT in the regulation of autophagy in iNs? CRISPRi



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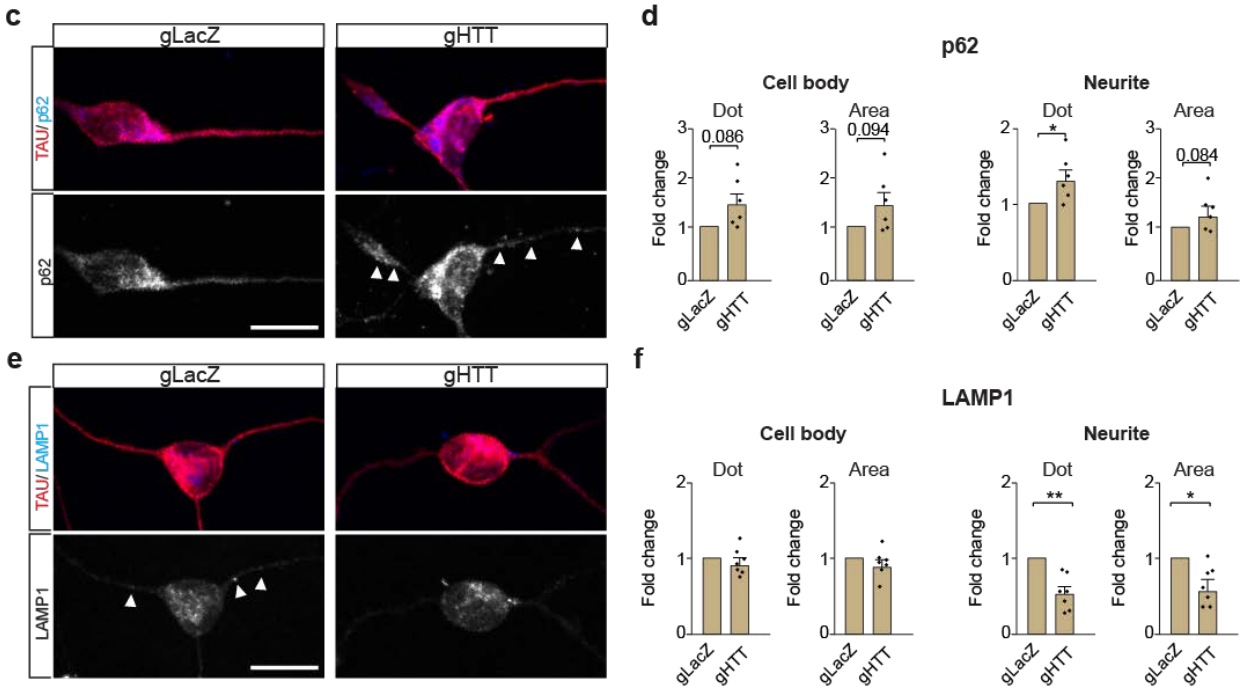
Silencing wtHTT (both healthy alleles) using CRISPRi: loss of function



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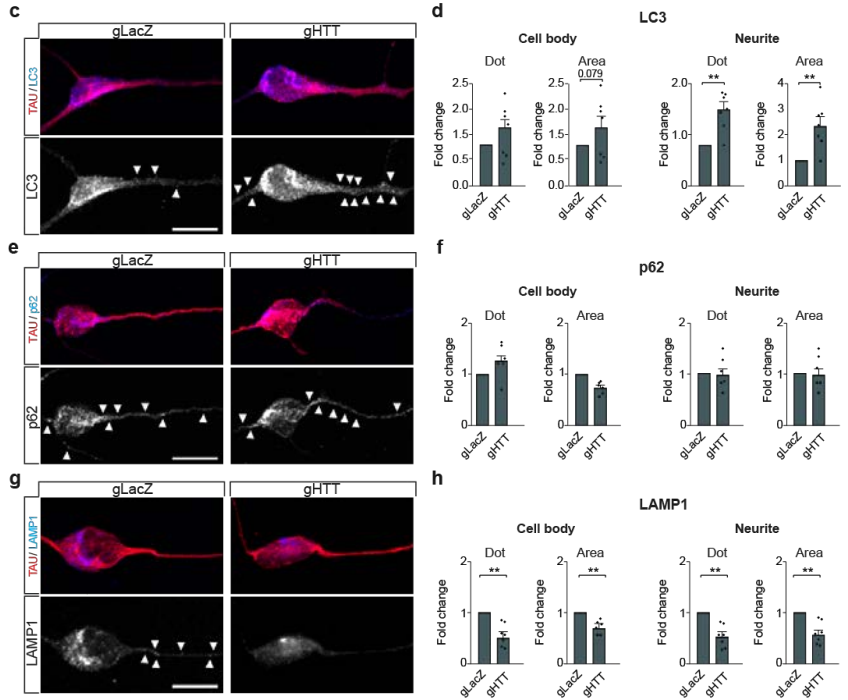
Silencing wtHTT & mHTT using CRISPRi: ratio and allele specificity is crucial



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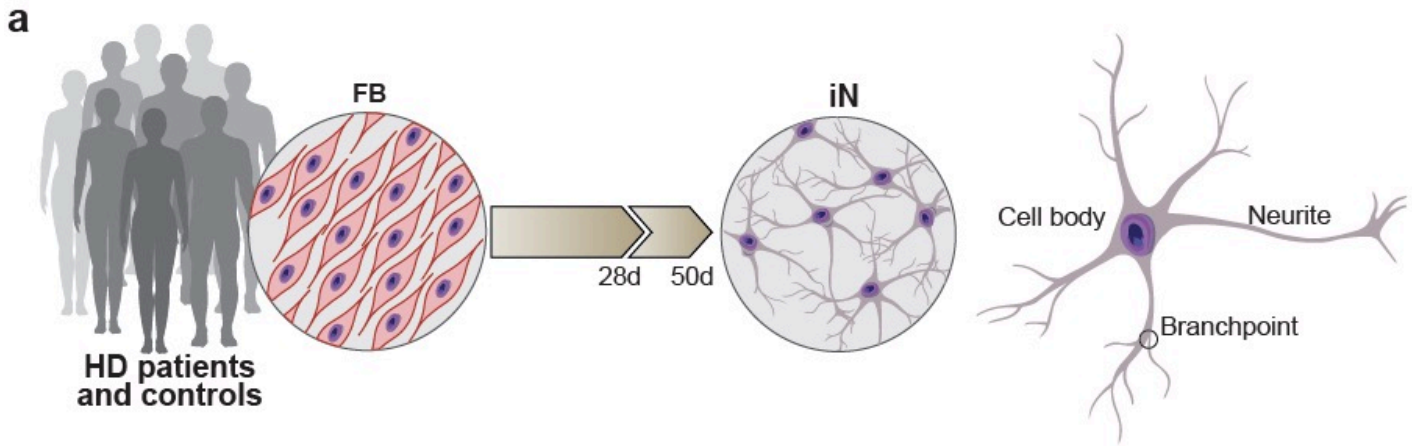
HCA
Neuronal profiling



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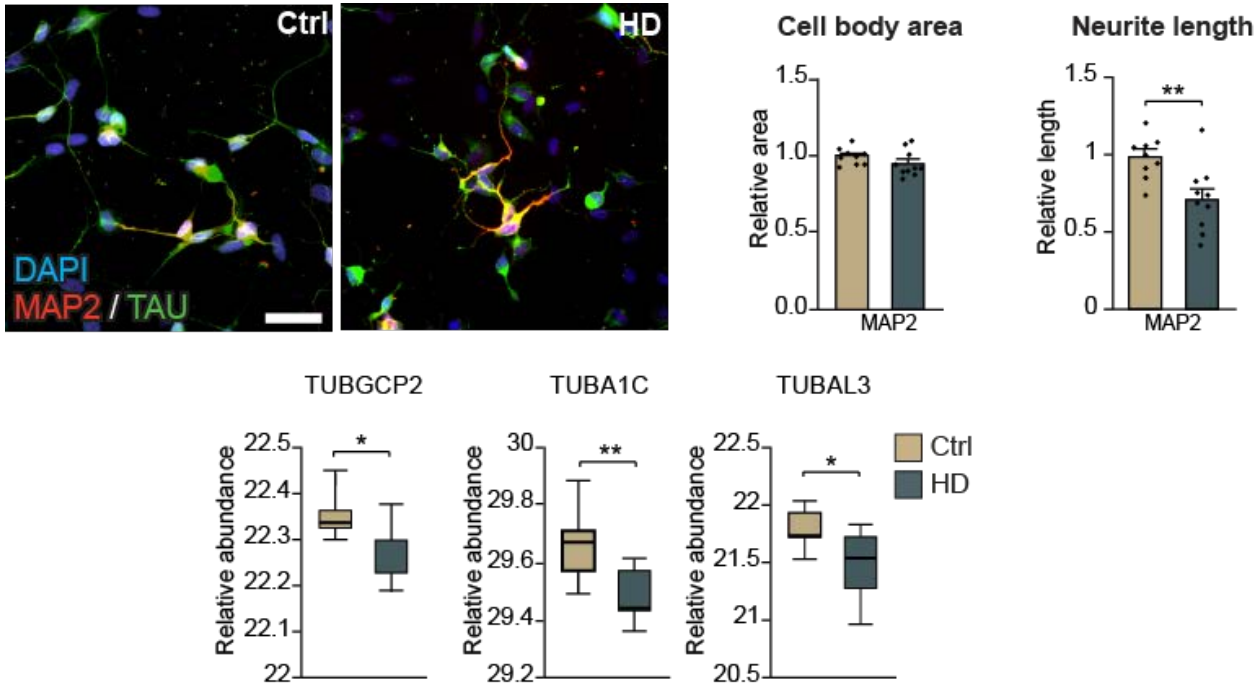
HD-iNs results in reduction in neurite complexity



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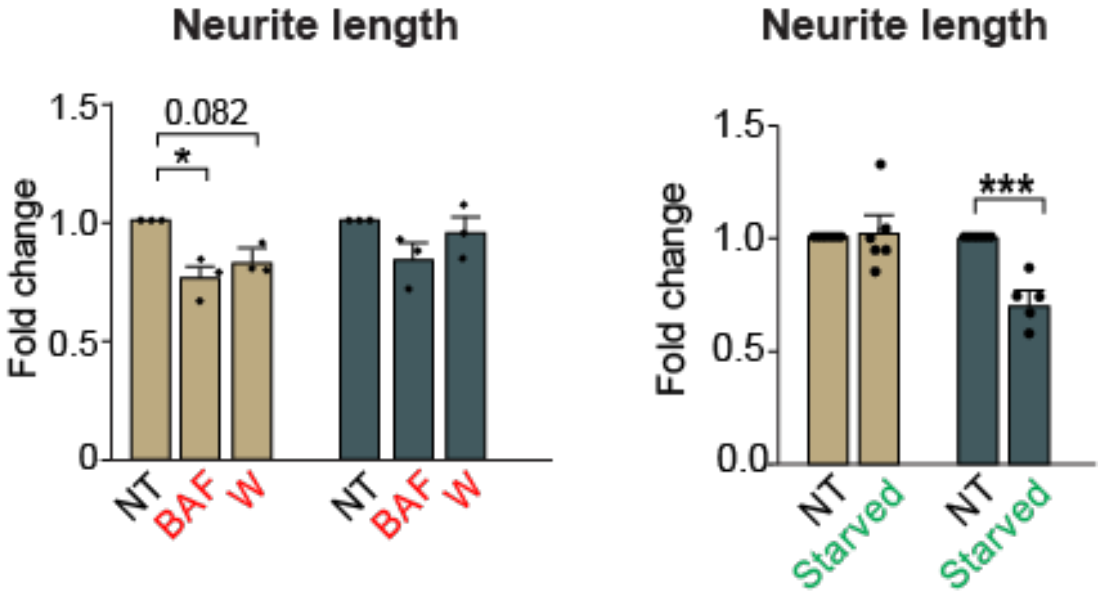
The autophagy impairment is linked to the reduced neurite complexity in HD-iNs



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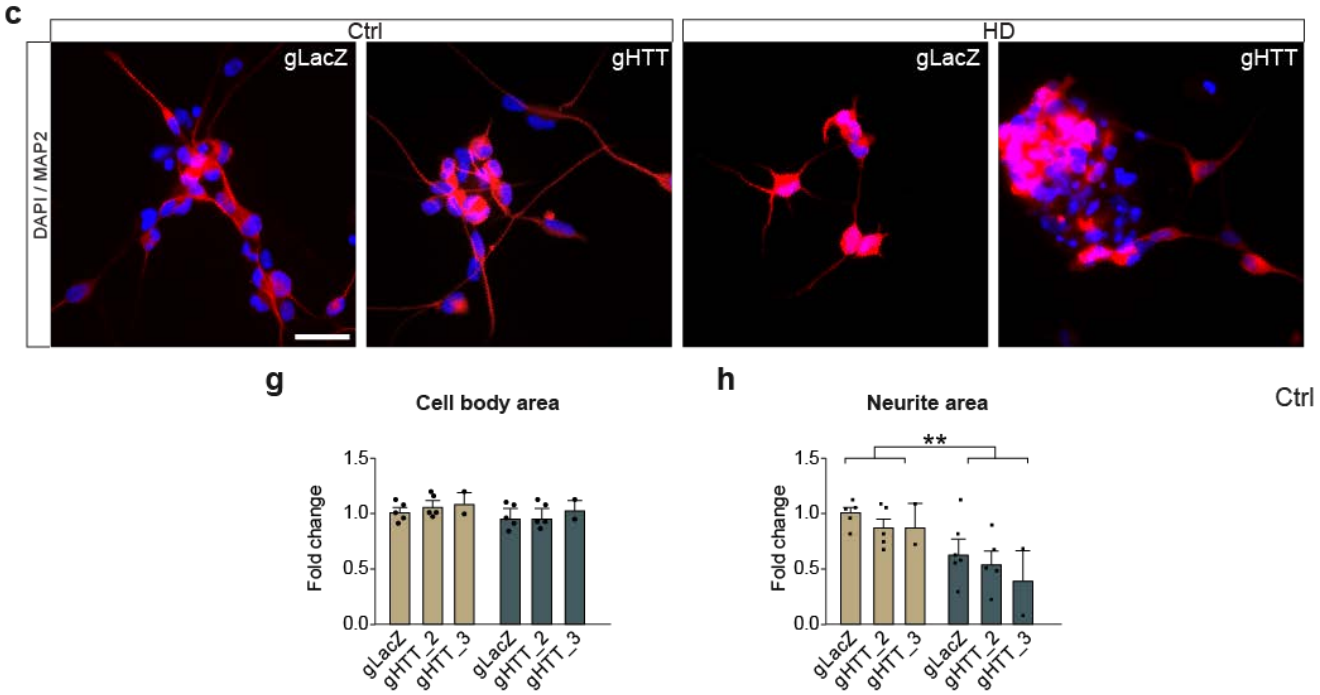
No rescue of disease-related phenotype HD neurons



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Discontinued ROCHE-ASO trial

nature

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NEWS | 05 May 2021 | Clarification 06 May 2021

Failure of genetic therapies for Huntington's devastates community

Hopes were high for drugs designed to lower levels of a mutant protein, but development has stalled.

- Roche has discontinued dosing in the Phase 3 GENERATION HD1 trial
- Intrathecal injections
- "no go" based on tominersen's potential benefit/risk profile
- Allele specific mHTT targeting could be the key!

Summary

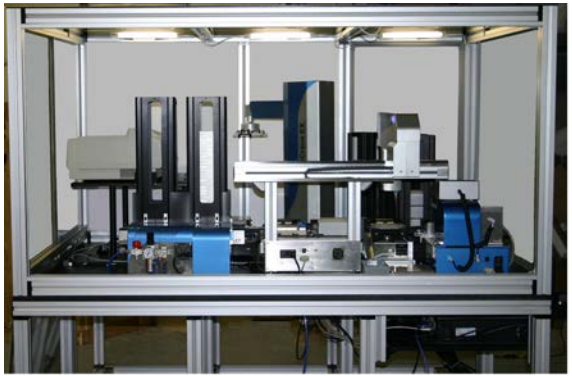
- Novel HD-iN model
- Posttranscriptional, cell type specific impairment in HD-iNs (AMPK, autophagy)
- Neurite specific autophagy impairement
- No mHTT aggregation, AGE
- We have developed a CRISPR-i system that efficiently silence HTT in several cell culture models – *including patient-derived neurons*
- *Gain of function/ loss of function!*
- Morphological differences in HD-iNs, linked to autophagy

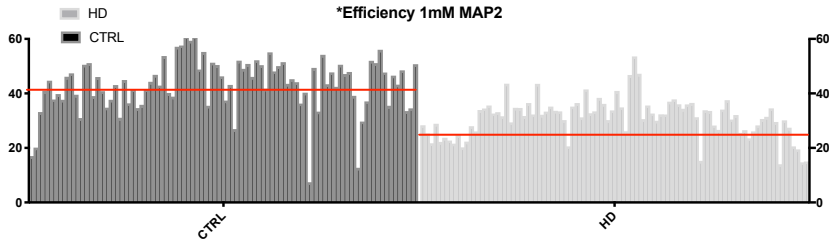
iN-based model of HD

- iNs are an excellent model to study late onset neurodegenerative disease:
  - retain the aging signature of the donor
- Allows for analysis of neurons obtained from many patients
- Range of CAG repeats: 39 – 50 (one 58)
- Robust protocol
- Compatible with High-content screening
- Allows for drug screening

iN-based model allows for drug screening

- Compatible with High-content screening
- Allows for automated high throughput drug screening (96-well plates)
- Can we identify drugs that reverse disease related phenotypes?
- Does such drugs work better in humans than those identified in other model systems?





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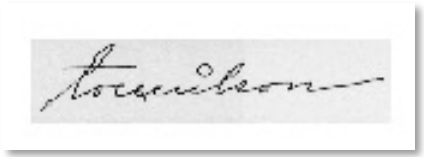
Fredrik Ek (Drug screening)


Multi
Park

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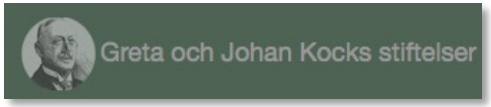
Greta och Johan Kocks stiftelser


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Thank you!