Department of Electron Microscopy

A. N. Belozersky Research Institute of Physico-Chemical Biology MSU

# Dynamic Properties of Microtubules: Investigation for Huntington's Disease Terranostics

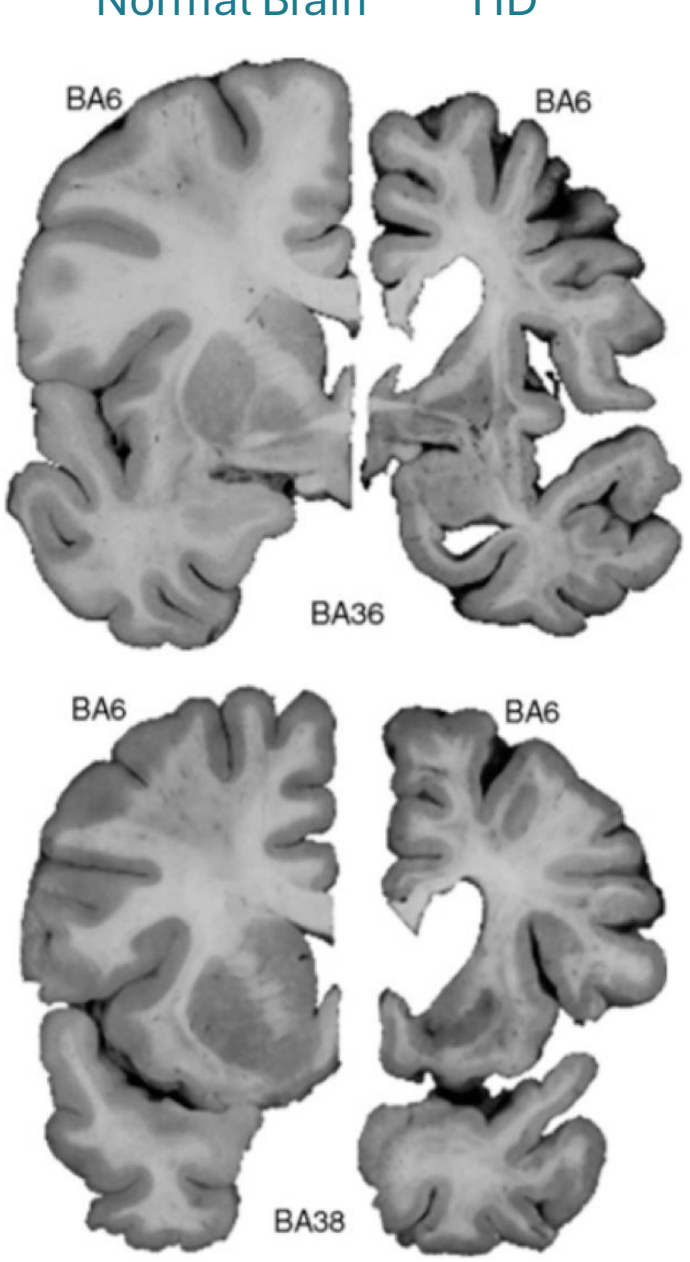
Speaker: Aleksandra Churkina (Taran)

Vyacheslav Pasko, Lilia Belikova, Alexandra Bogomazova, Mariya Lagarkova, Irina Alieva

AYSS, Dubna, 2024

# Huntington disease (HD)

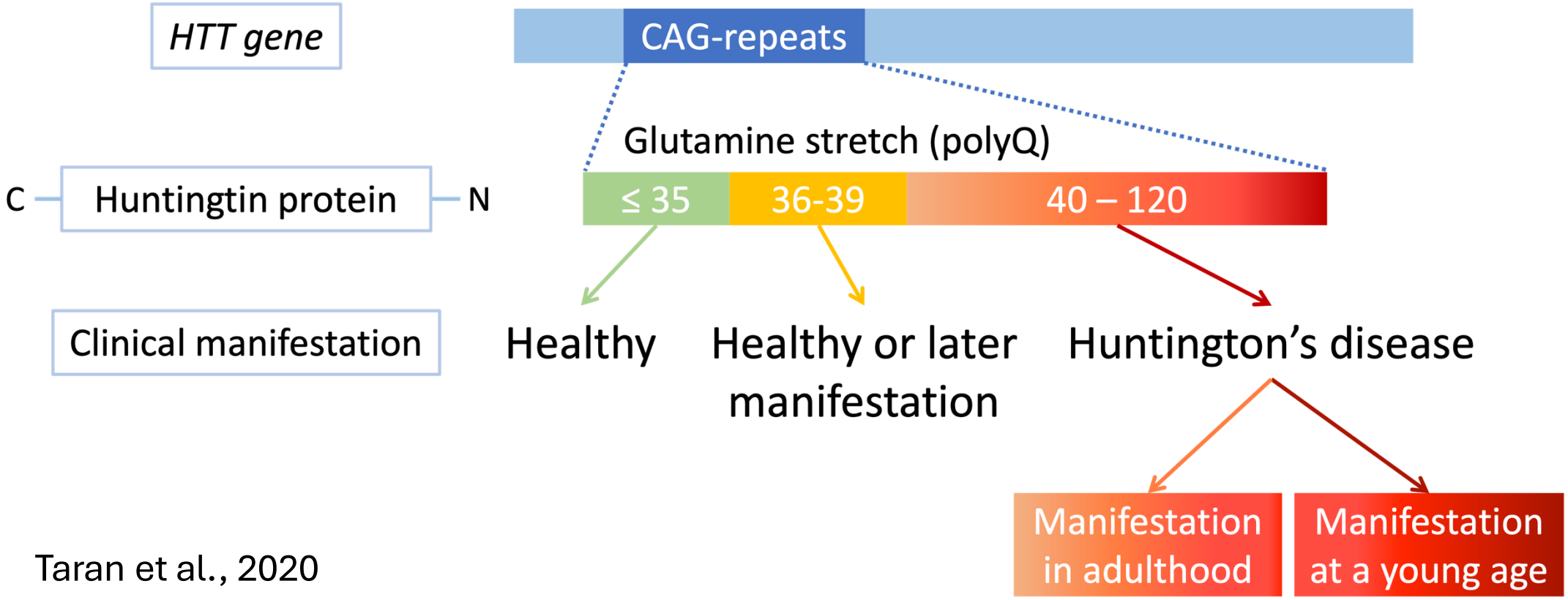
- hereditary neurodegenerative disease
- onset usually in middle age (30-50 years)
- clinical features: mental disorders and choreic hyperkinesis
- striatum atrophy (projection neurons death)
- at a late stage the cerebral cortex atrophy




Vonsattel et al., 2008

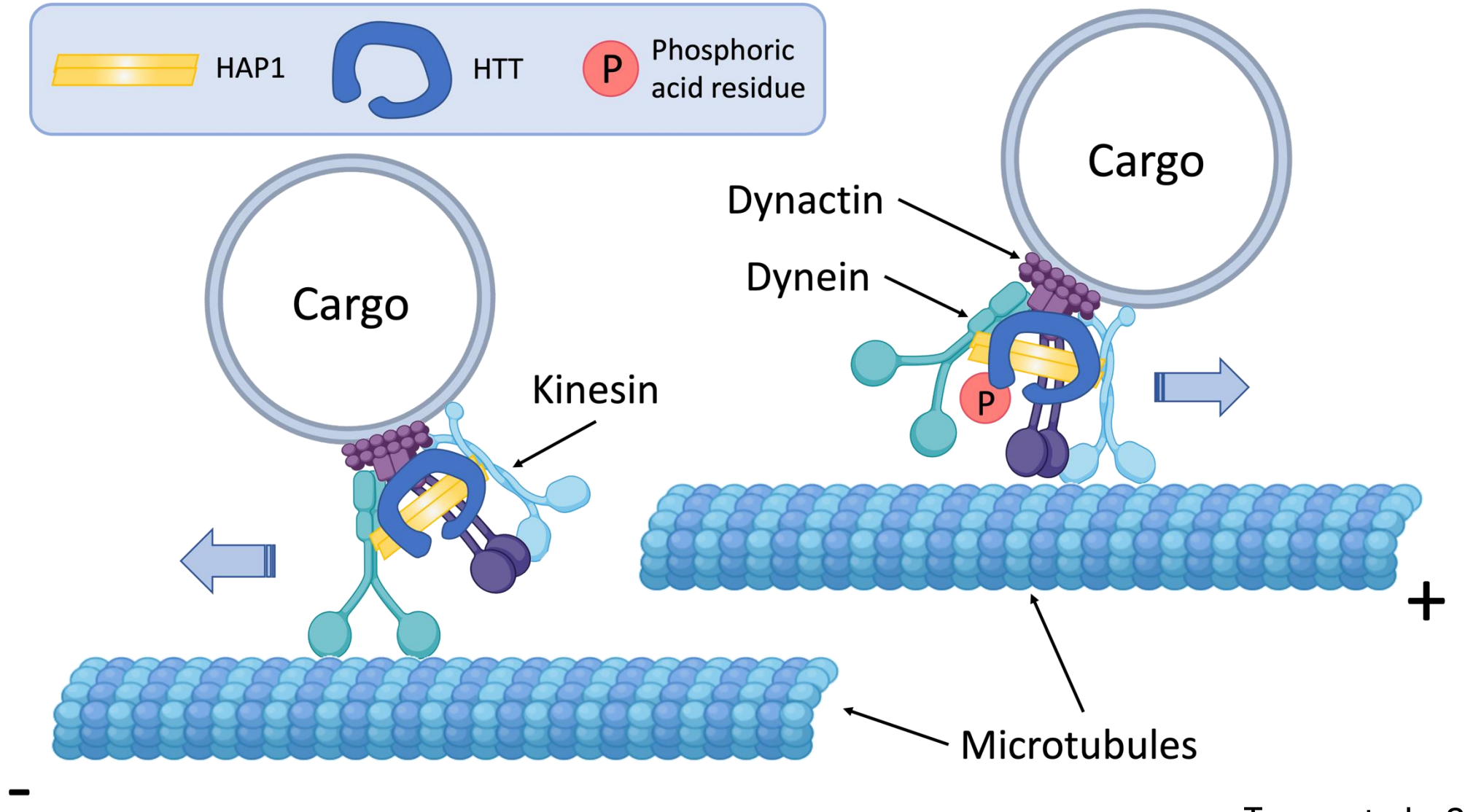
# Cause of Huntington's disease

- CAG codon multiplication (glutamine amino acid) in the HTT gene, which encodes the 350-kDa huntingtin protein (HTT)
- the onset age and the disease manifestation degree depend on the CAG-repeats number



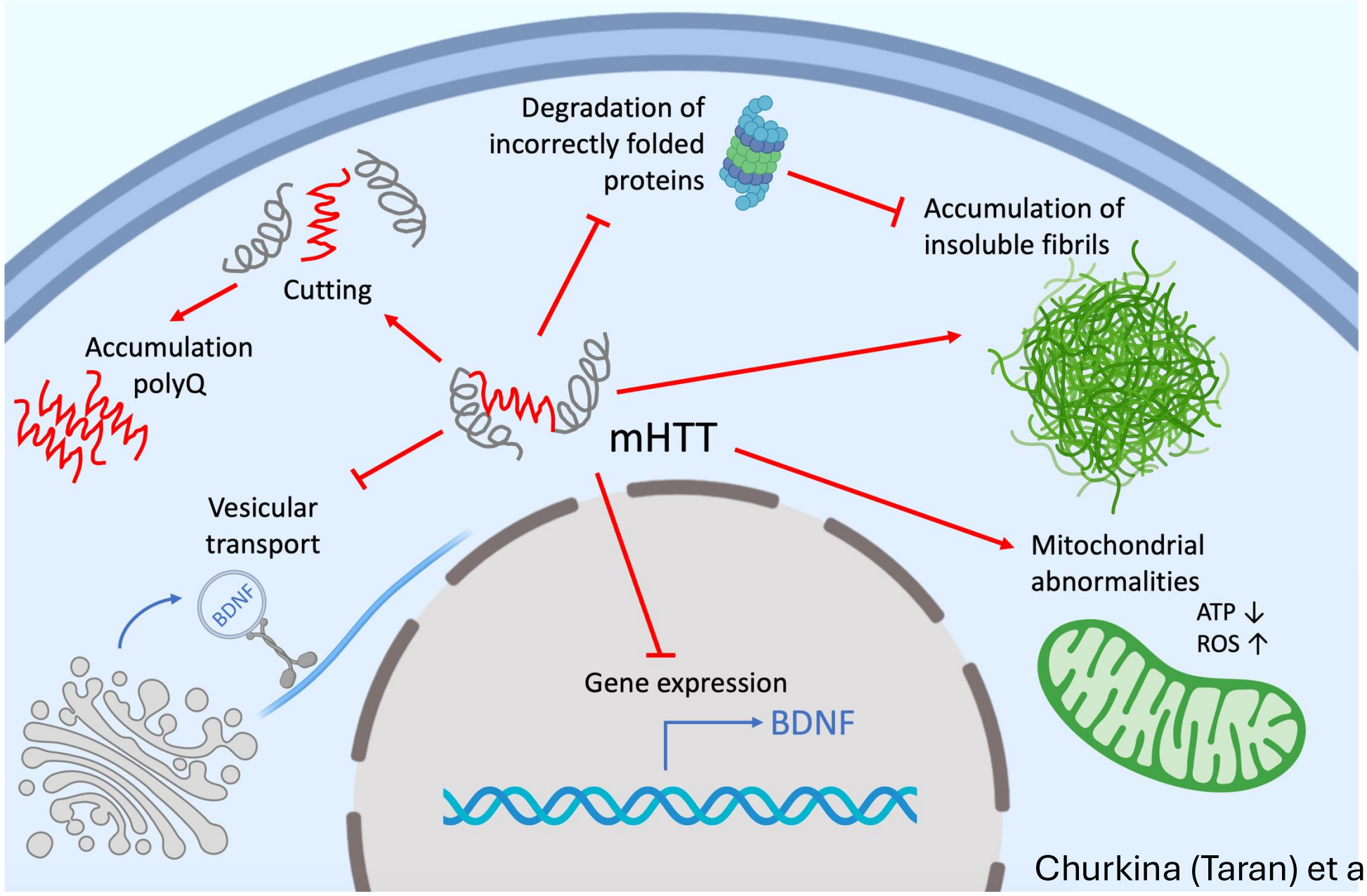

Taran et al., 2020

# The supposed functions of huntingtin protein (HTT)




Taran et al., 2020

# Putative mechanisms of mutant huntingtin toxicity



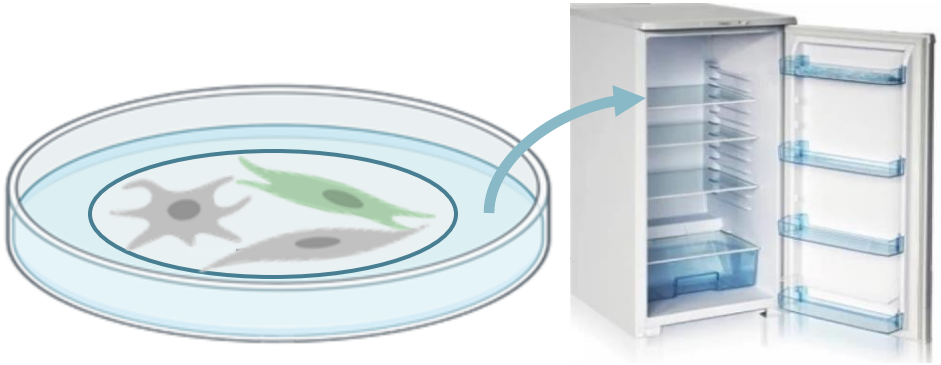

Churkina (Taran) et al., 2020

# Model systems

1. Cultured *in vitro* fibroblasts obtained from the HD patients skin with a different CAG-repeats number in the HTT gene
2. Cultured neurons derived from IPSC (induced pluripotent stem cells)

| Donor | Number of CAG repeats | Age at the time of biopsy | Sex |
|---|---|---|---|
| HD42 | 42 | 45 | ♂ |
| HD47 | 47 | 46 | ♀ |
| HD76 | 76 | 17 | ♂ |

# The microtubules repolymerization after their complete disassembly



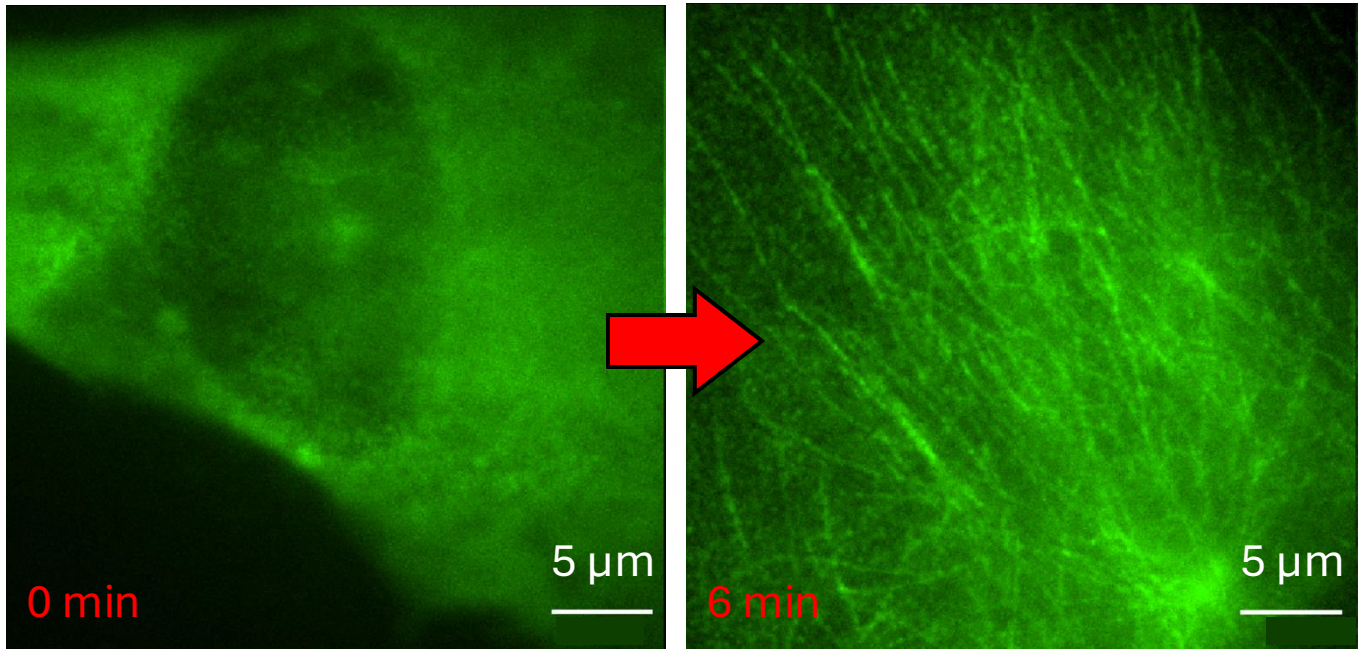
Incubation for 2 hours at +4°C

Complete MTs network destruction

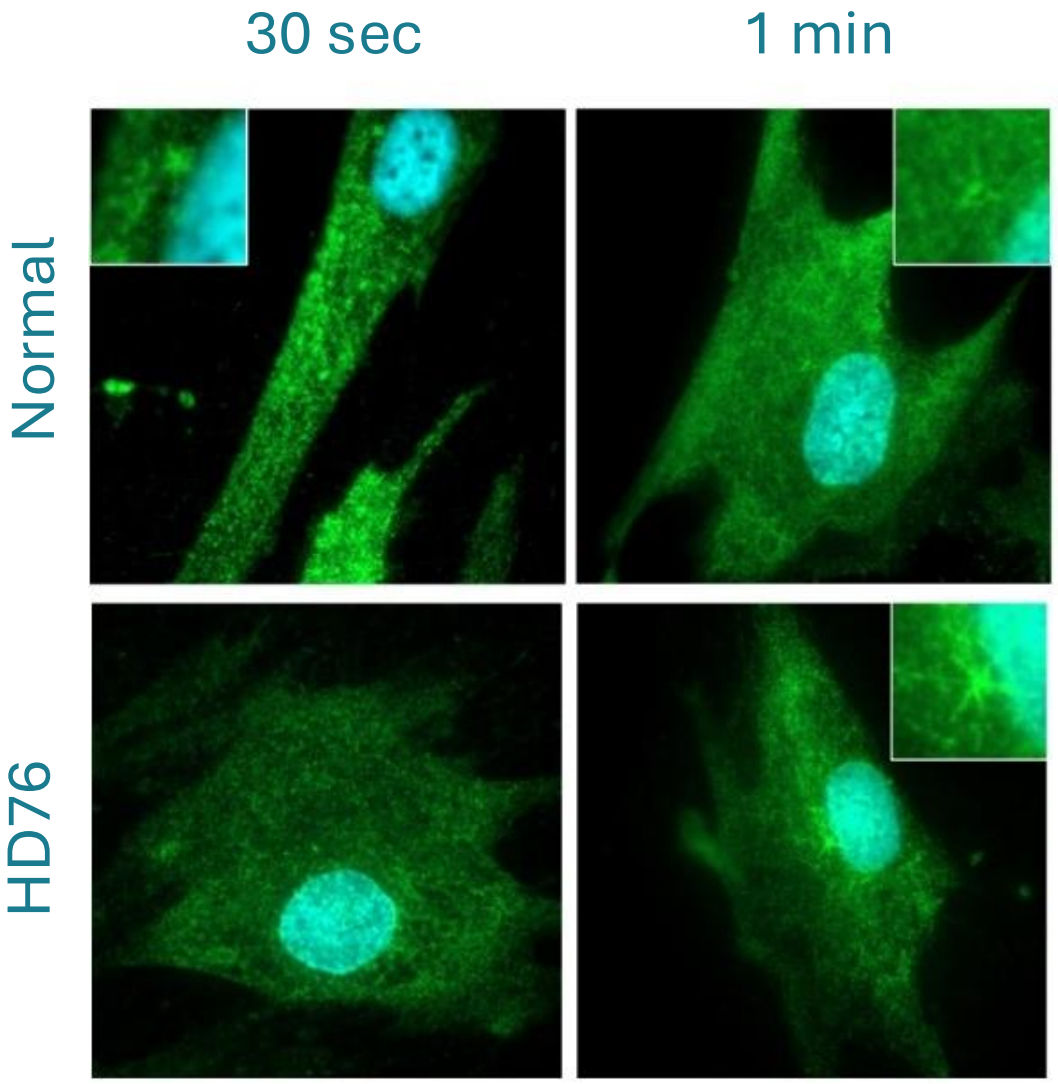
Network recovery at +25°C for 30 seconds, 1, 2, 4, 6, 8, 10 and 30 minutes

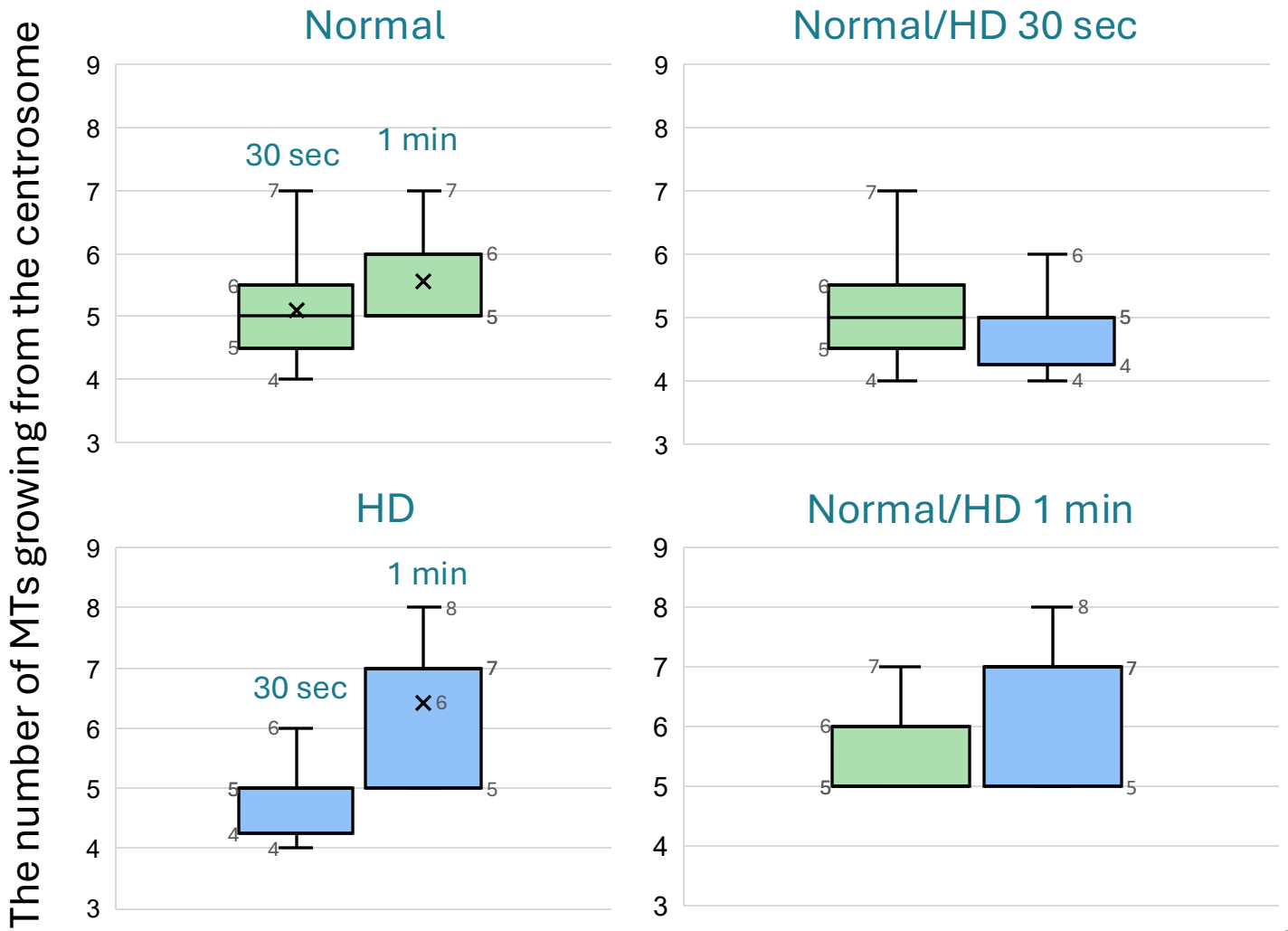
Complete microtubules network disassembly

Network recovery



# The microtubules system recovery begins quickly and in the early stages does not differ in the HD patients cells and normal

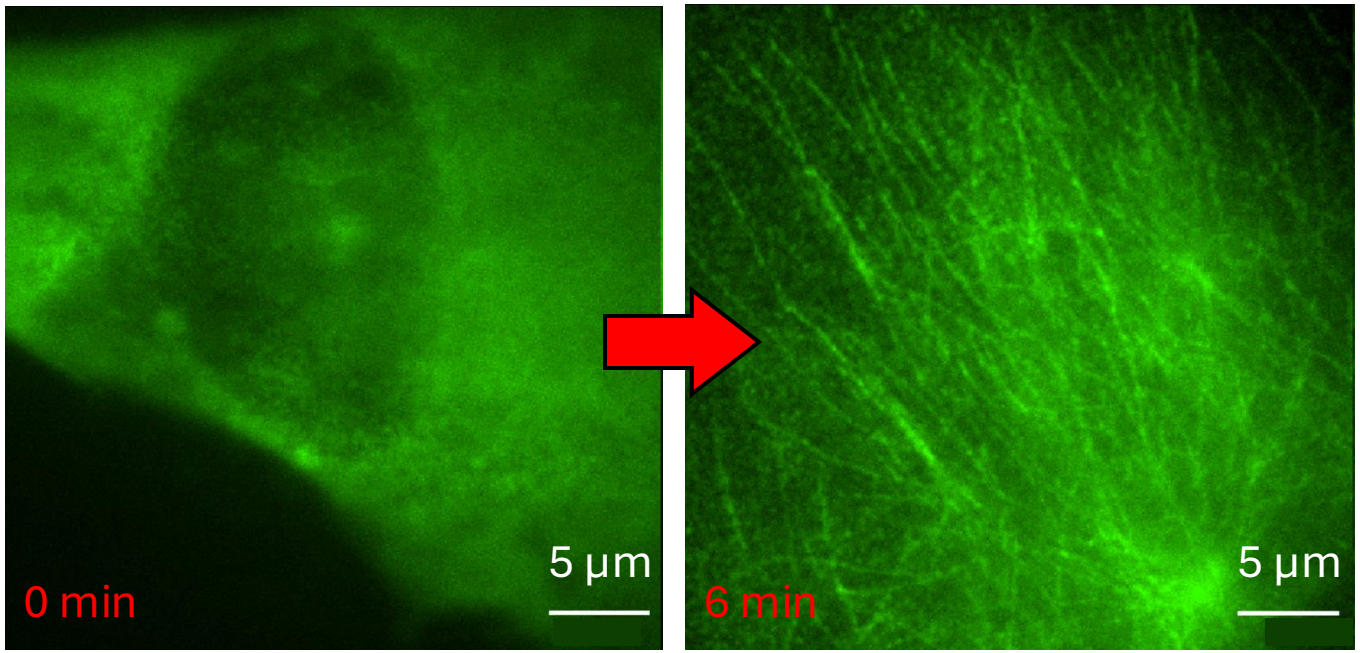


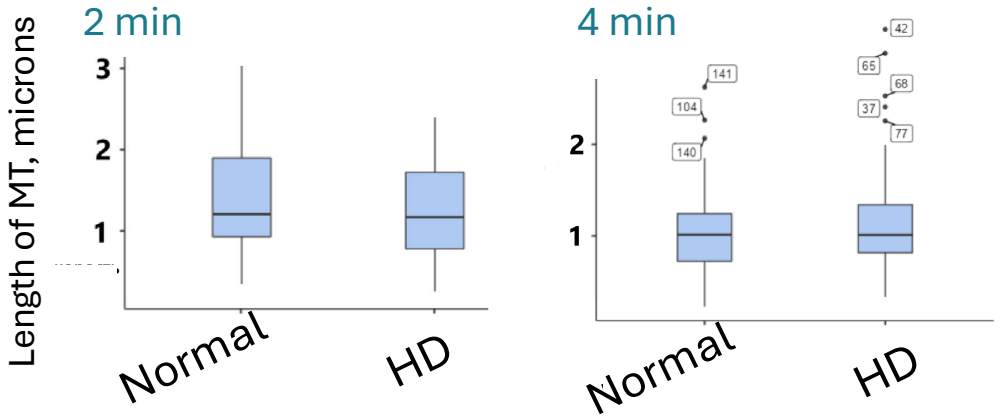



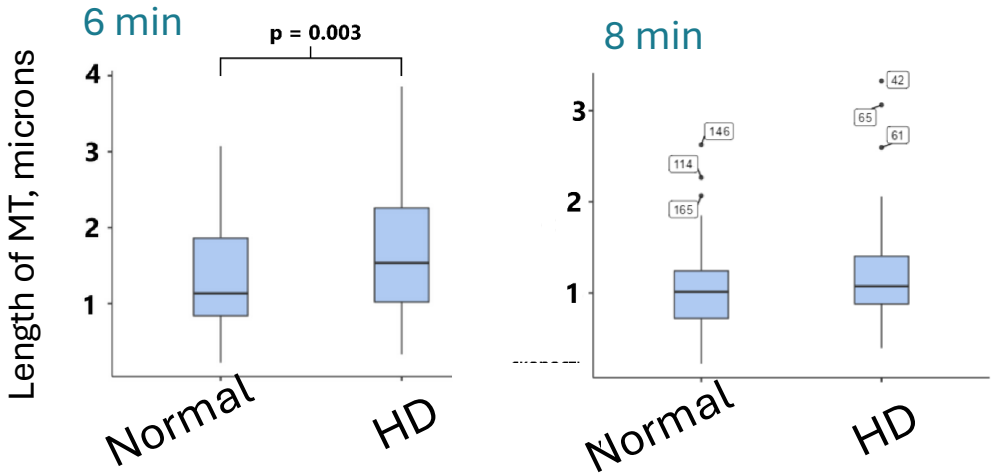
# The average length of the recovering microtubules after their complete disassembly is higher in HD patients

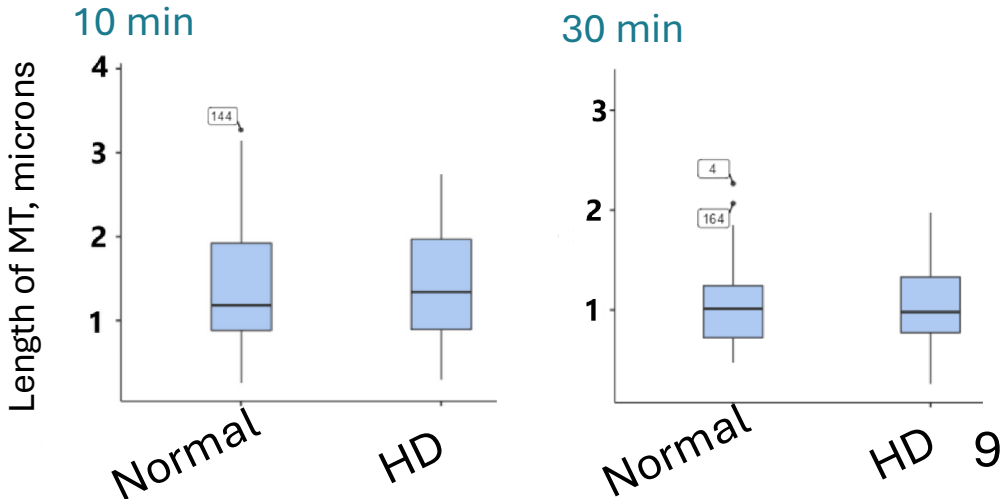
MTs network complete disassembly

Network restoration



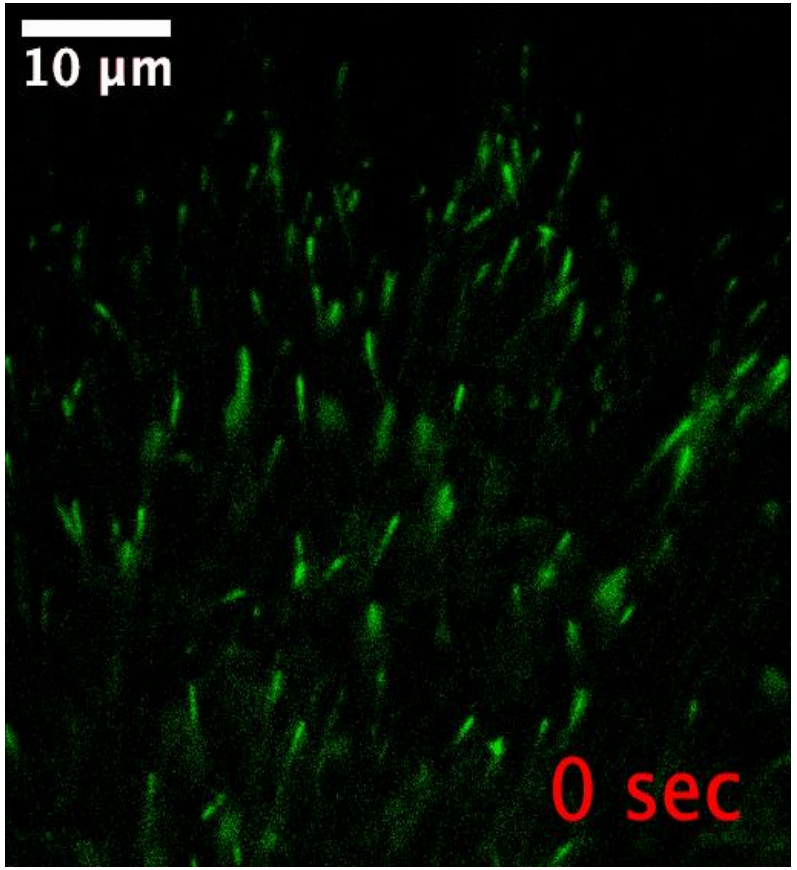



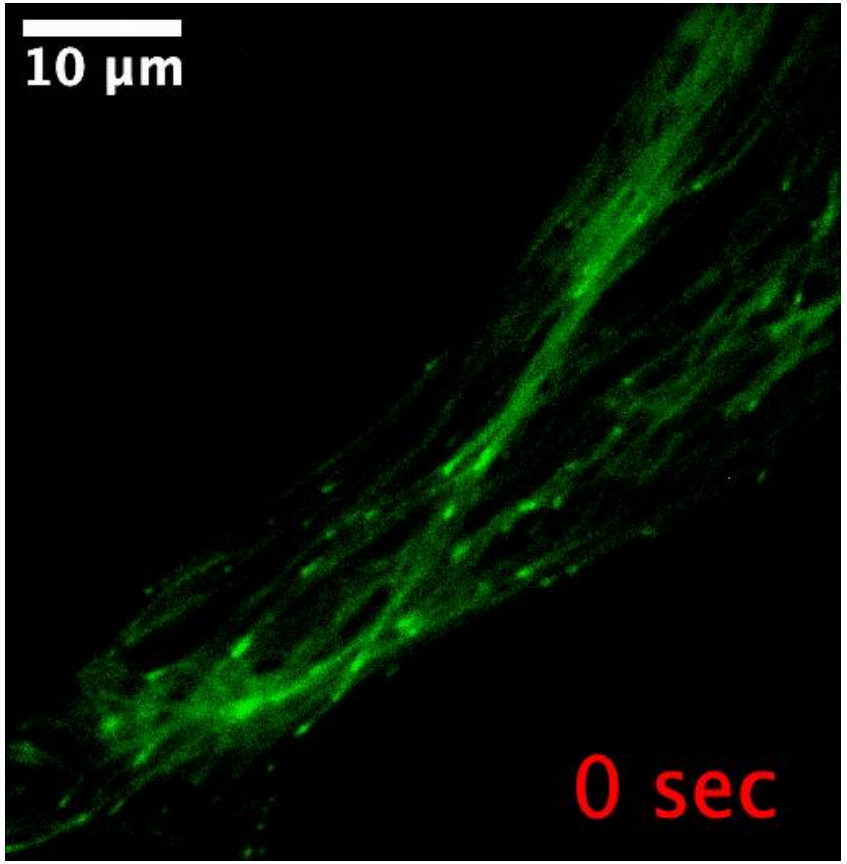



# Microtubule dynamics analysis in functionally different fibroblast zones

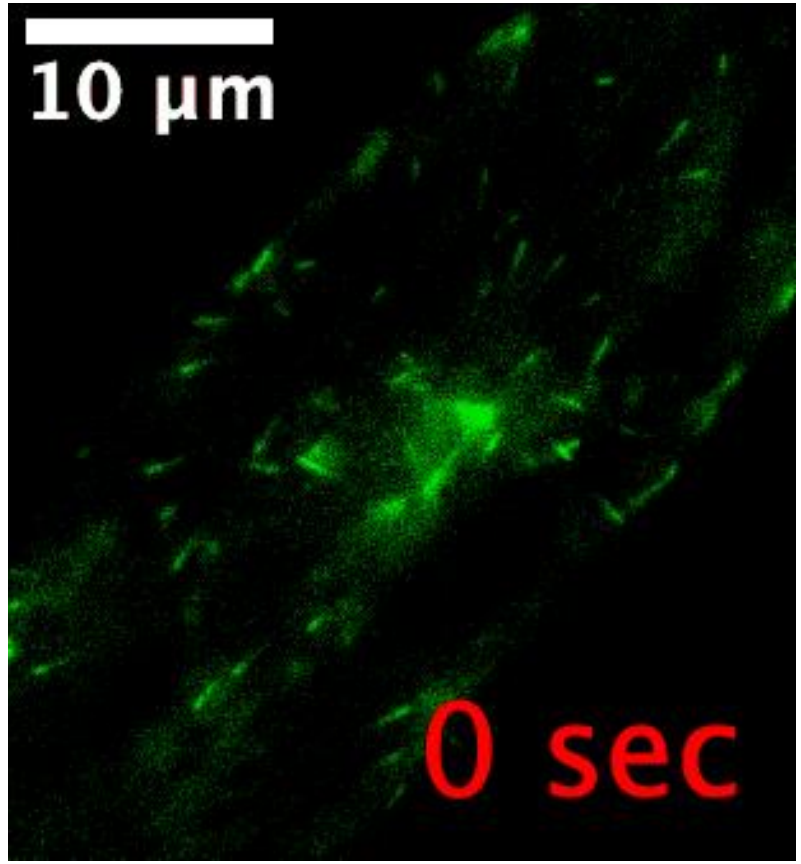
The leading edge




Tail




Central part

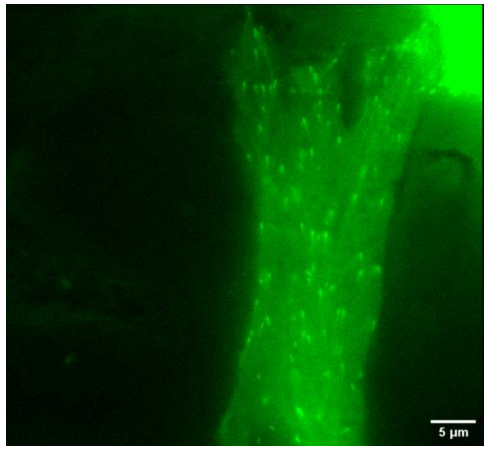


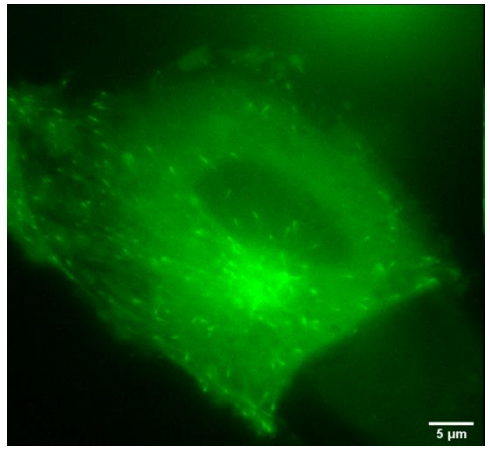

GFP-EB3 label in various areas of transfected primary skin fibroblasts

# Microtubules dynamic properties in different parts of cultured skin fibroblasts

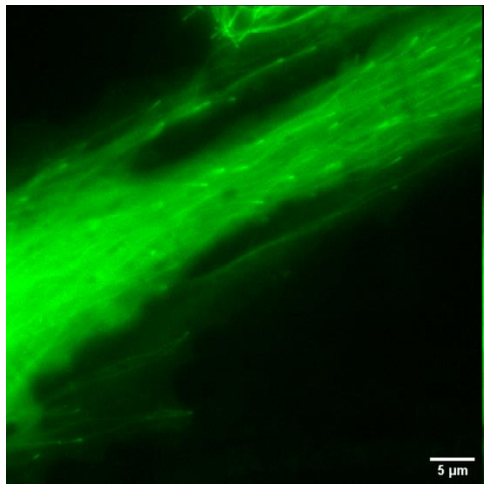
Central part

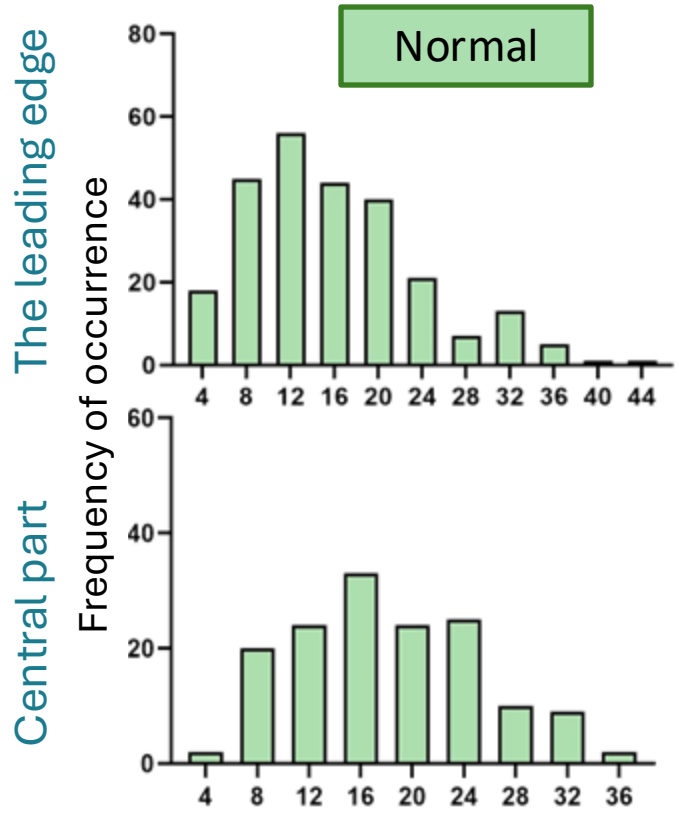
The leading edge

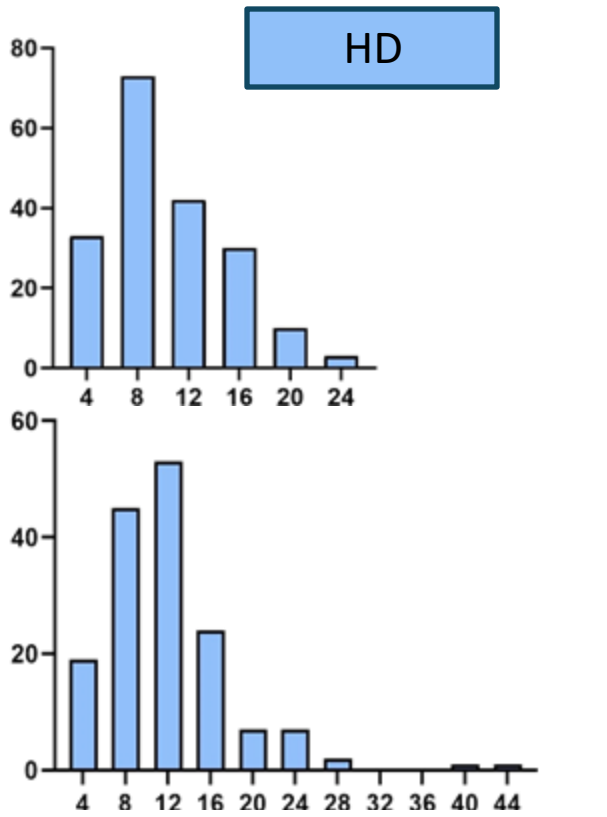



Tail

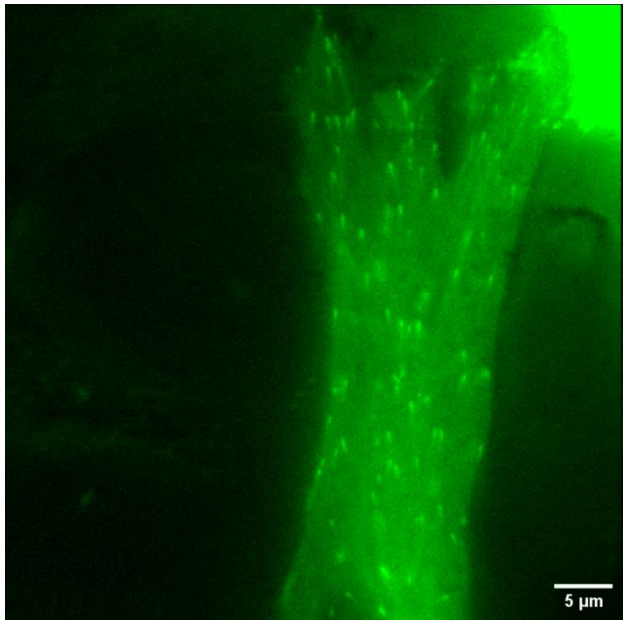


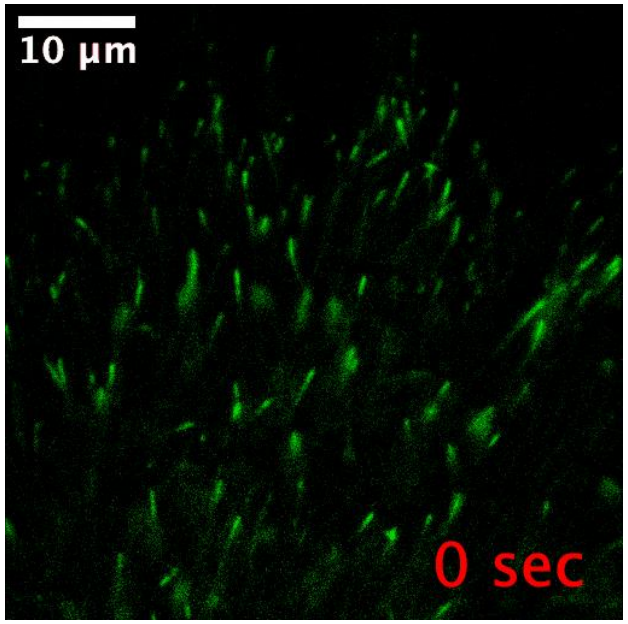



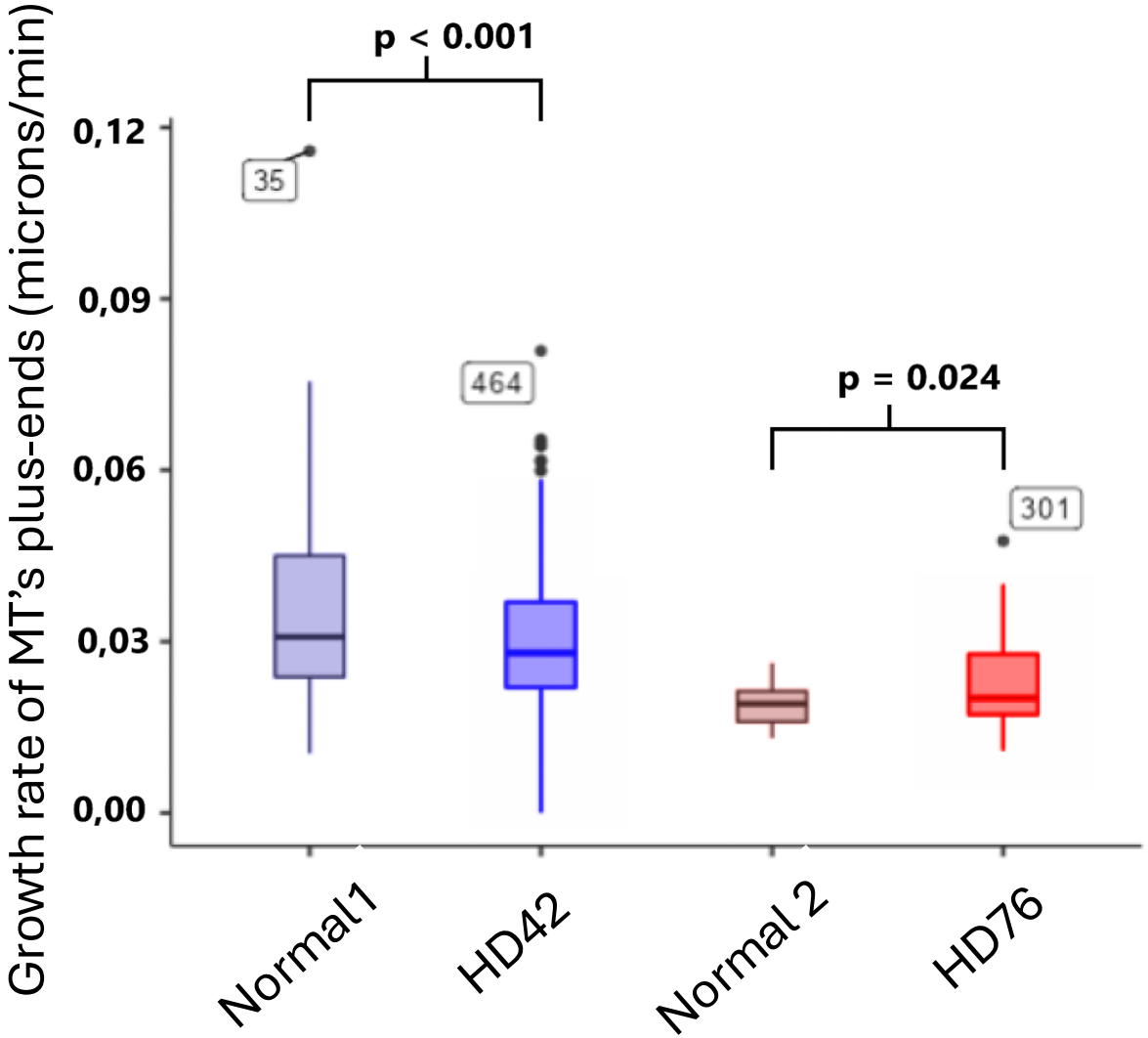

| Cell part | Average growth rate (microns/min) | | Max and min growth rate | | The ratio of pioneer MTs (%) | | Pause frequency | |
|---|---|---|---|---|---|---|---|---|
| | Normal | HD | Normal | HD | Normal | HD | Normal | HD |
| Central | 19,42±0,69 | 12,29±0,43 | Max 36,72<br>Min 5,46 | Max 43,26<br>Min 3,84 | 8,94 | 1,18 | 0,5±0,28 | 0,8±0 |
| The leading edge | 16,52±0,49 | 10,78±0,32 | Max 43,26<br>Min 3,84 | Max 23,52<br>Min 5,46 | 10,12 | 0 | 0,8±0,46 | 2,2±0,28 |
| Tail | 12,52±0,38 | 11,76±0,3 | Max 36,48<br>Min 5,46 | Max 38,1<br>Min 5,46 | 1,44 | 0,46 | 0,46±0,11 | 2±0,87 |

# Microtubules dynamic properties on the leading edge of HD patients skin fibroblasts depends on the CAG repeats number

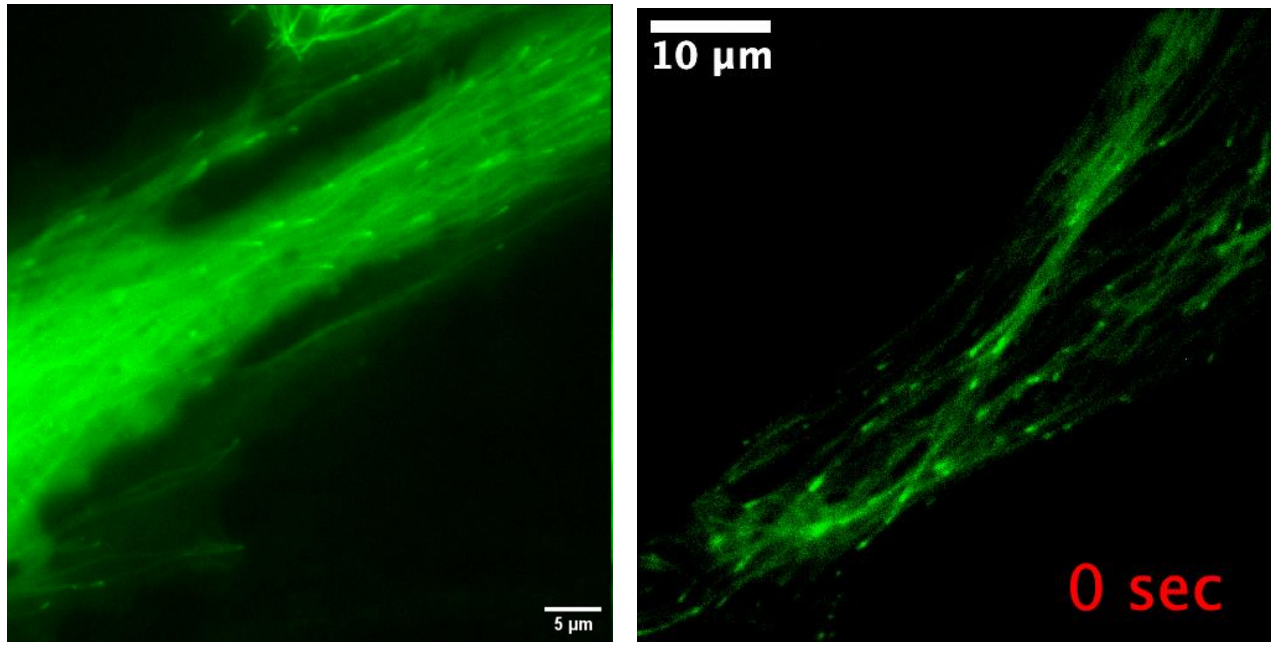


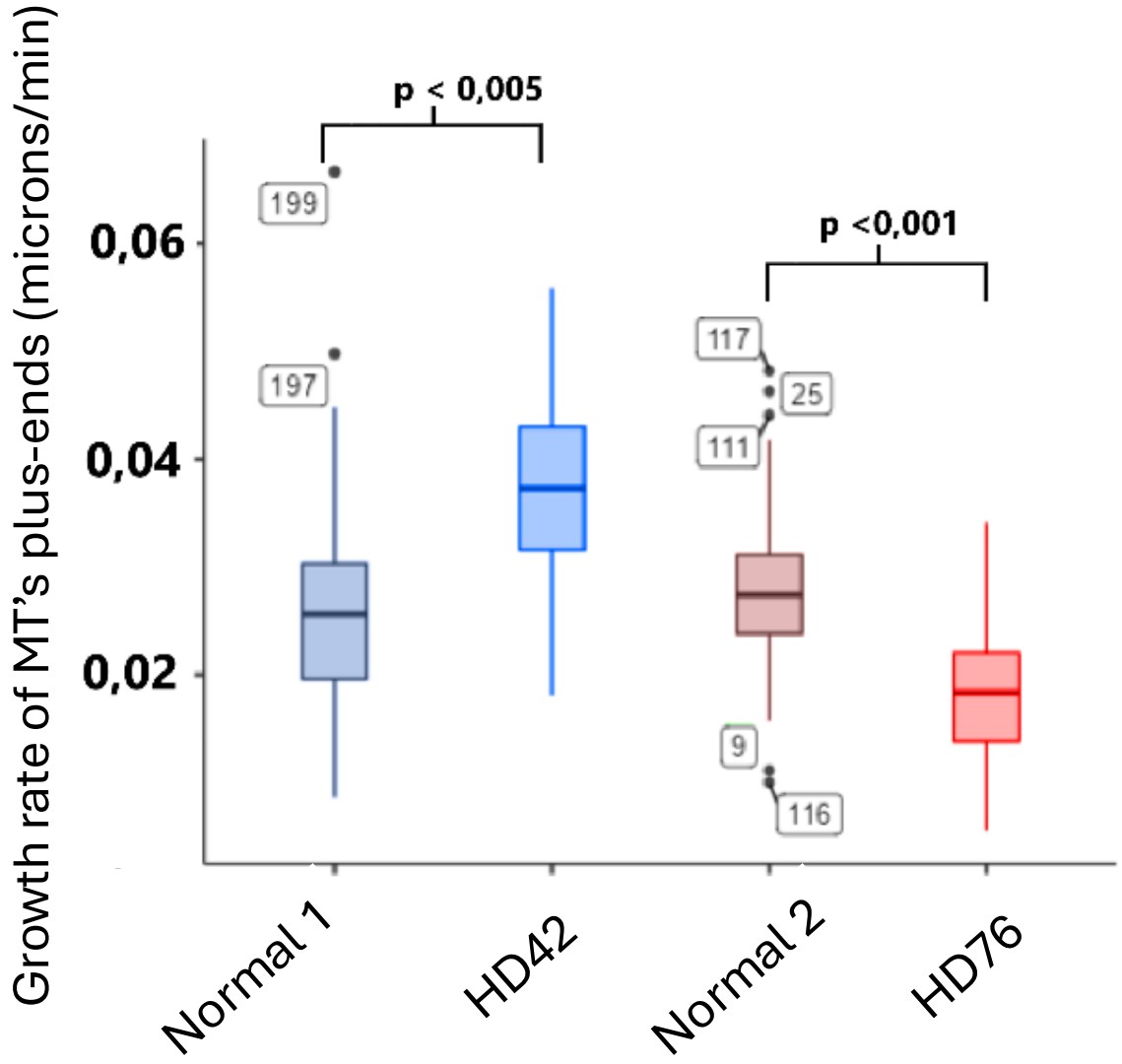

- In HD42 the MT's process fragments growth rates were reduced, while the number of pioneer MTs increased compared to the control
- HD76 has an increase in dynamic parameters in comparison with the control



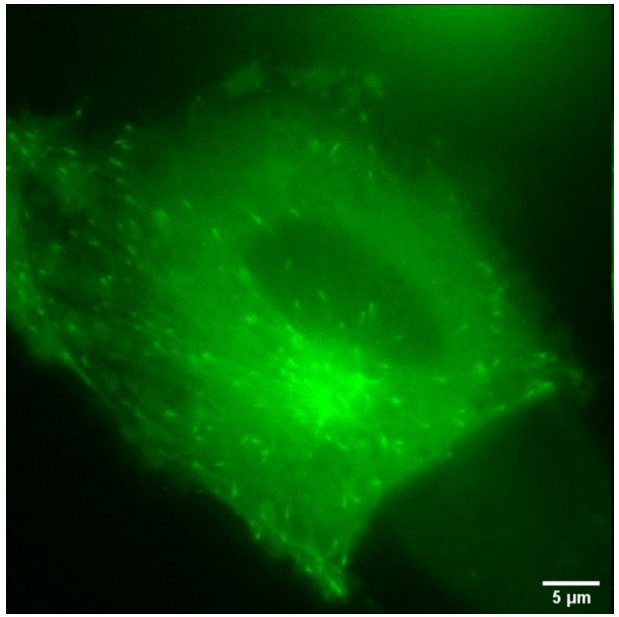
# Microtubules dynamic properties on the tail of HD patients skin fibroblasts depends on the CAG repeats number

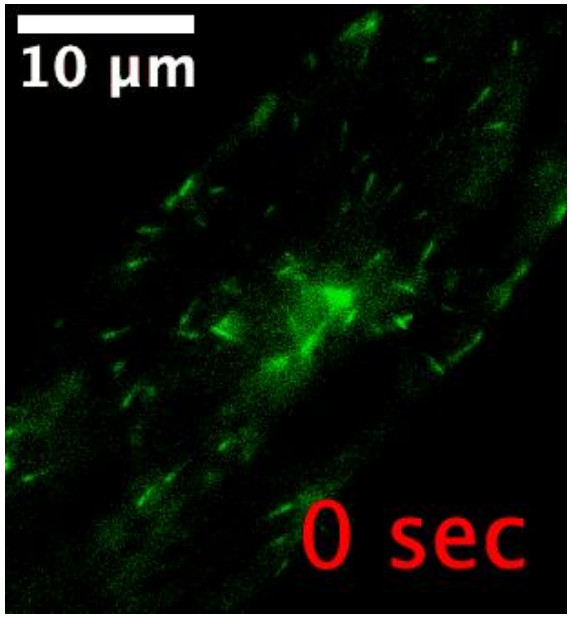



- HD42 has an increase in the MT's dynamic parameters
- HD76 has a decrease in the process MT's fragments growth rates, while there are no pioneer MTs

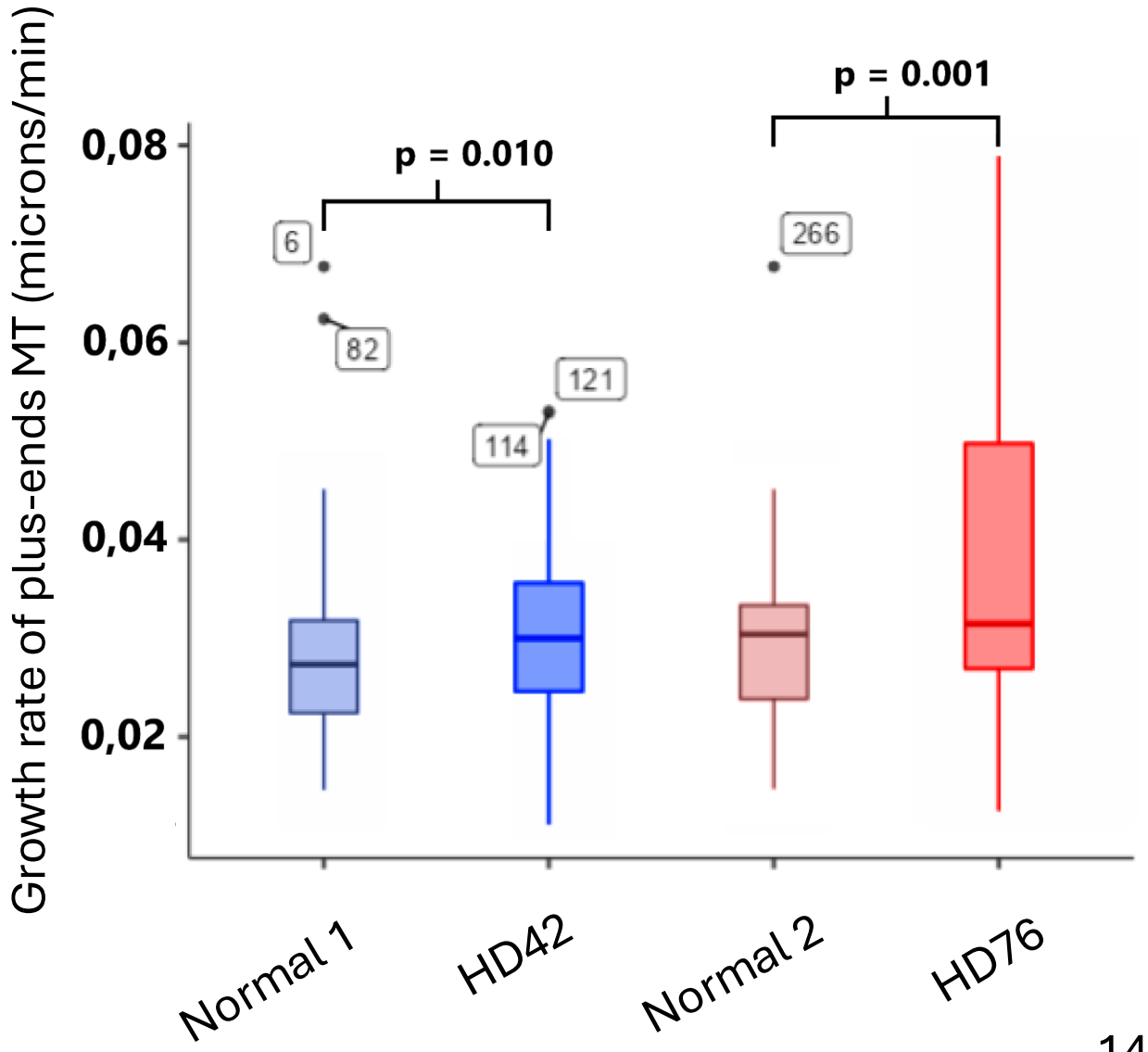


# Microtubules dynamic properties in the central part of HD patients skin fibroblasts does not depend on the CAG repeats number



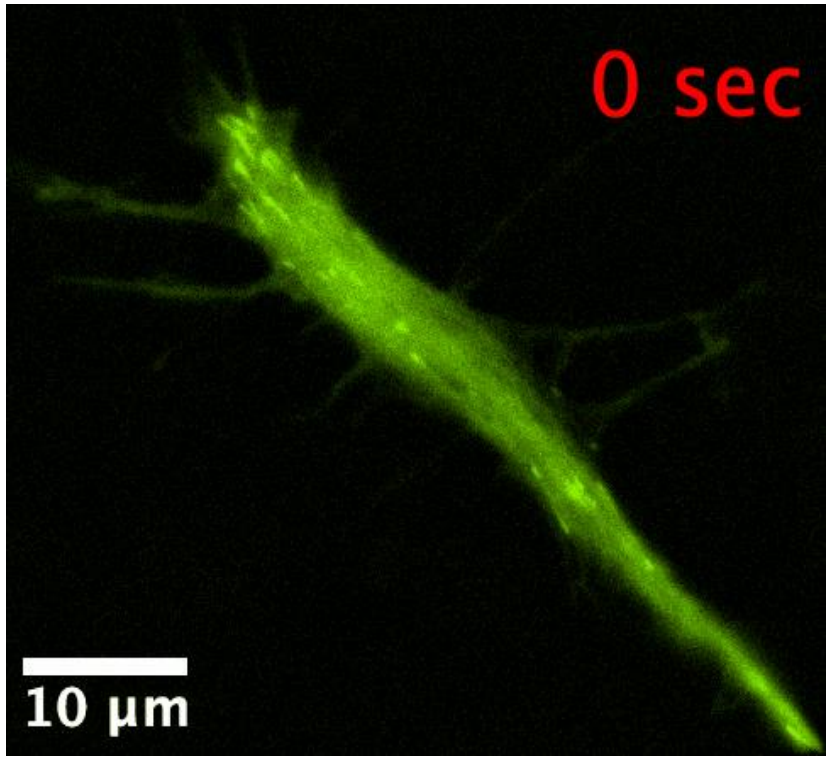




- Both HD42 and HD76 have an increase in the MT's process fragments growth rates
- HD76 lacks pioneer MTs

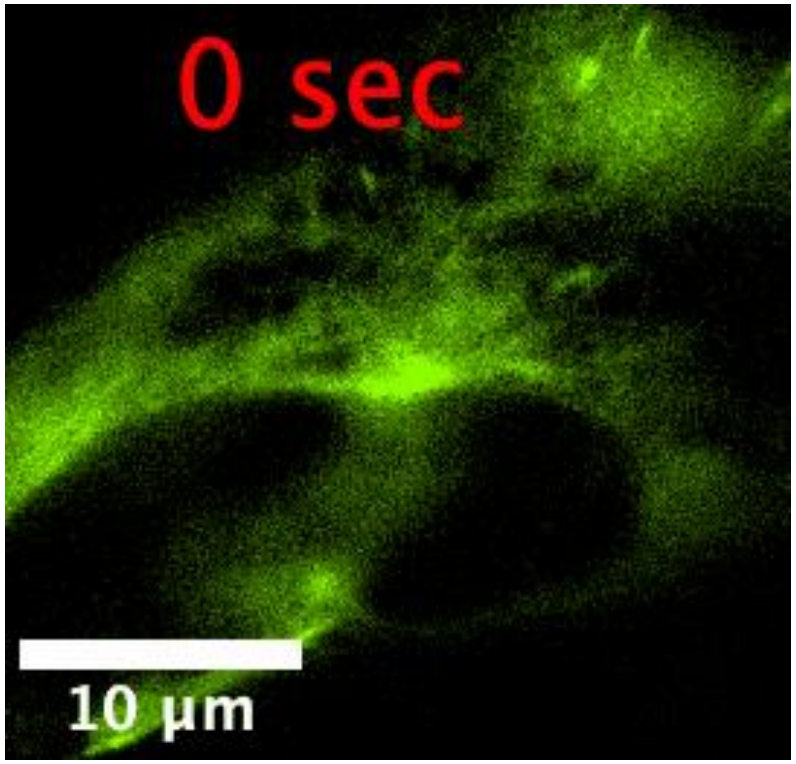


# Microtubule dynamics analysis in functionally different neuron zones

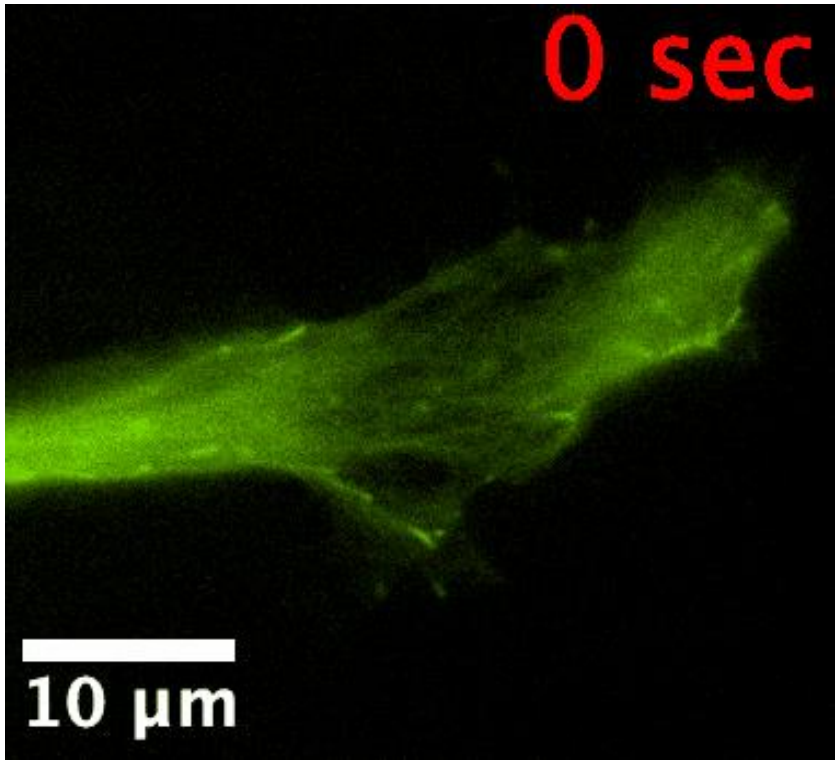
Dendrite




Central part (body)

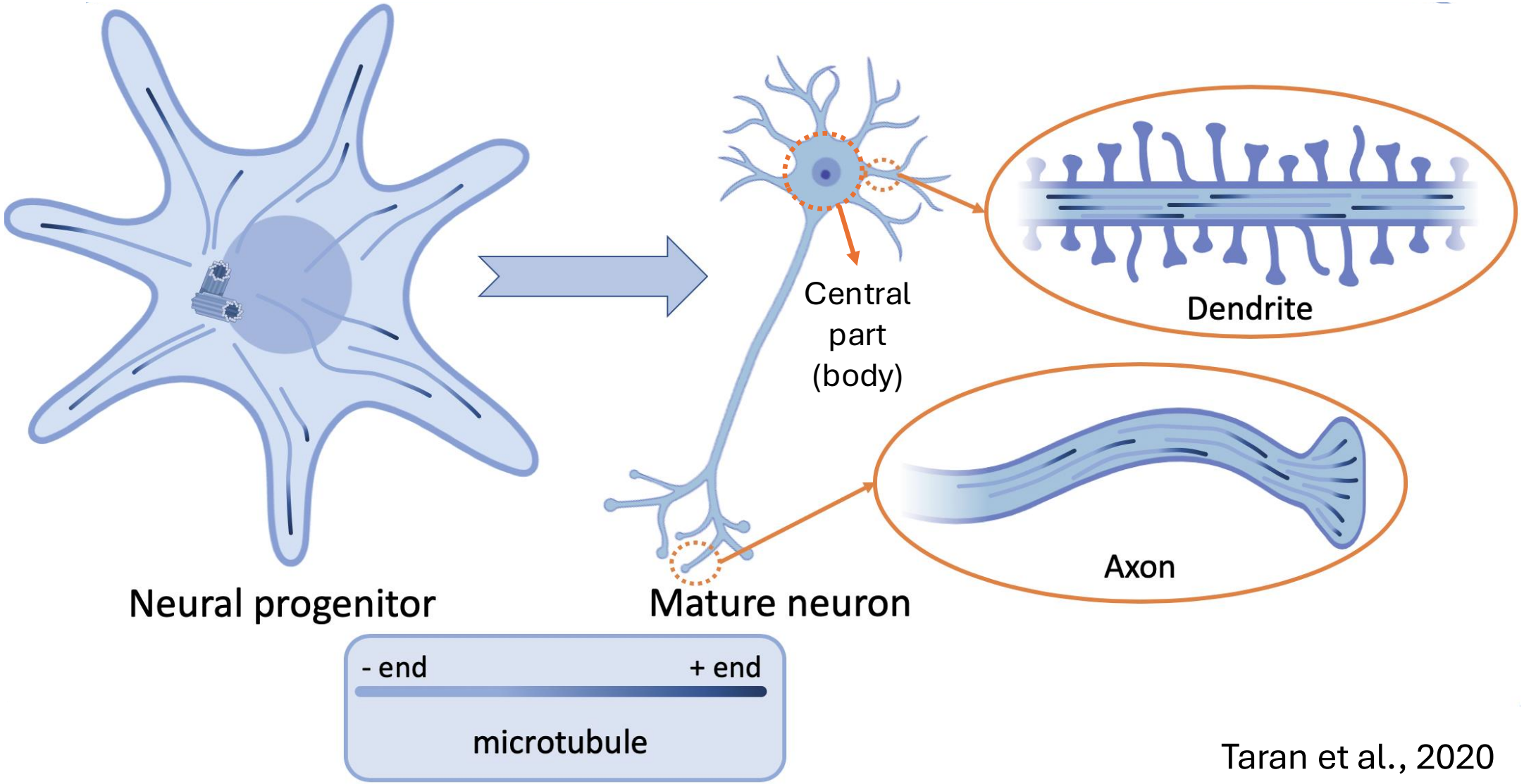



Axon



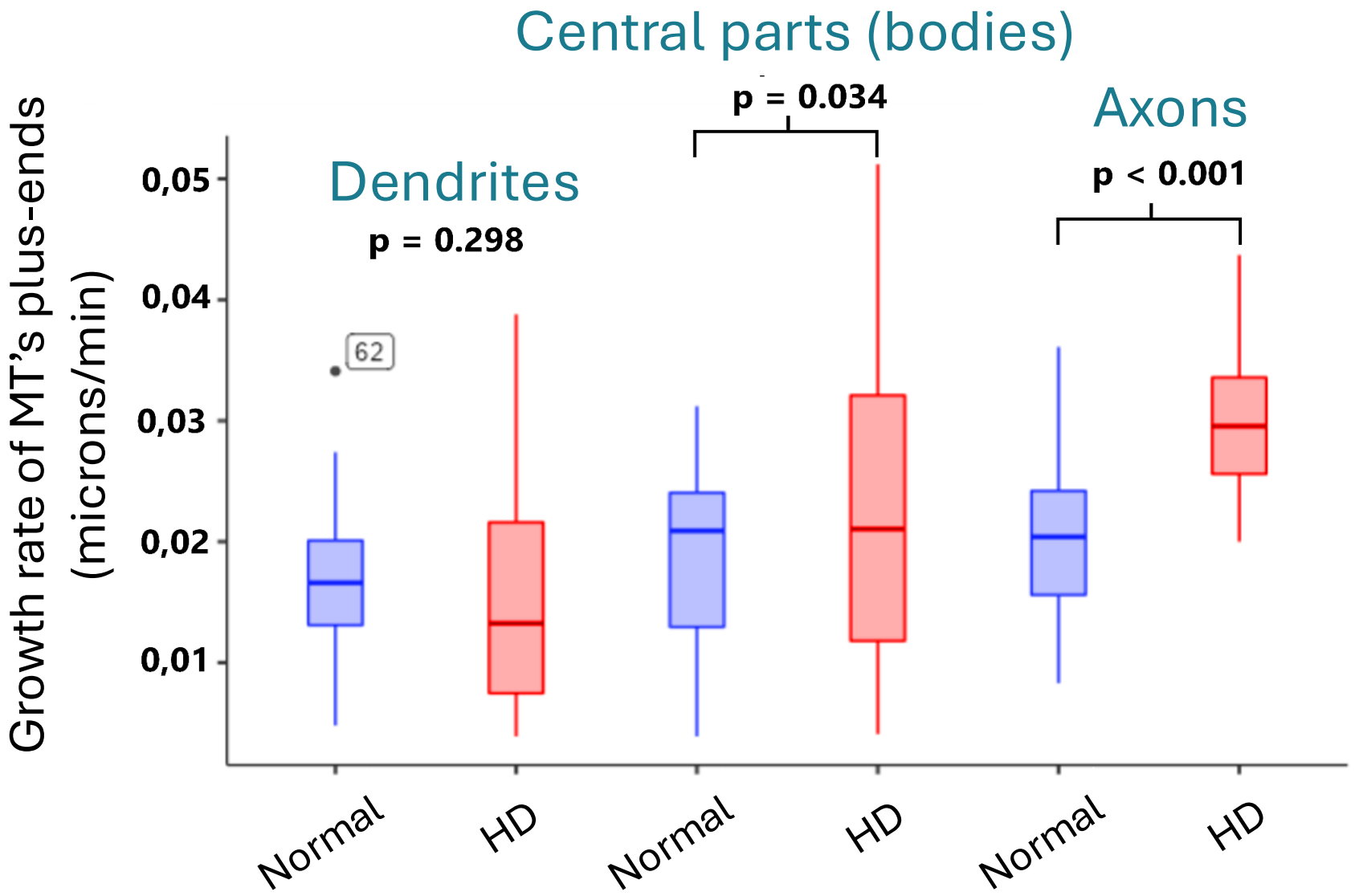

GFP-EB3 label in various areas of transfected neurons

# The microtubule growth direction differs in axons and dendrites of neurons




Taran et al., 2020

# Microtubules growth rate increases in the axons and the bodies but not in the dendrites of HD patients neurons



# Conclusions

1. The dynamic properties of the MT's network in the HD patients cells differ from those of healthy donors.
2. The MT's dynamic properties depend on the CAG-repeats number.

# Perspectives for terranostics

- These results for the first time made it possible to evaluate *in vitro* differences in the MT's dynamic properties in cultured fibroblasts and neurons of HD patients and healthy donors
- The effect of mutant HTT on the MT dynamics properties can be considered as approach for HD patients diagnostics and possible therapy

# Acknowledgements

Scientific supervisor DSc I. B. Alieva

**A. N. Belozersky Research Institute:**

DSc V. B. Dugina

DSc I. I. Kireev

PhD S. A. Golyshev

PhD M. A. Chelombitko

S. V. Lavrushkina

A. S. Shakhov

V. I. Pasko

**U. M. Lopukhin Federal Research and Clinical Center of Physical-Chemical Medicine of Federal Medical Biological Agency:**

Prof. DSc M. A. Lagarkova

A. E. Kharitonov

O. A. Zubkova

L. D. Belikova

https://app.biorender.com/ website was used to create the illustrations

The work is supported by the MSU Development Program (PNR 5.13.)