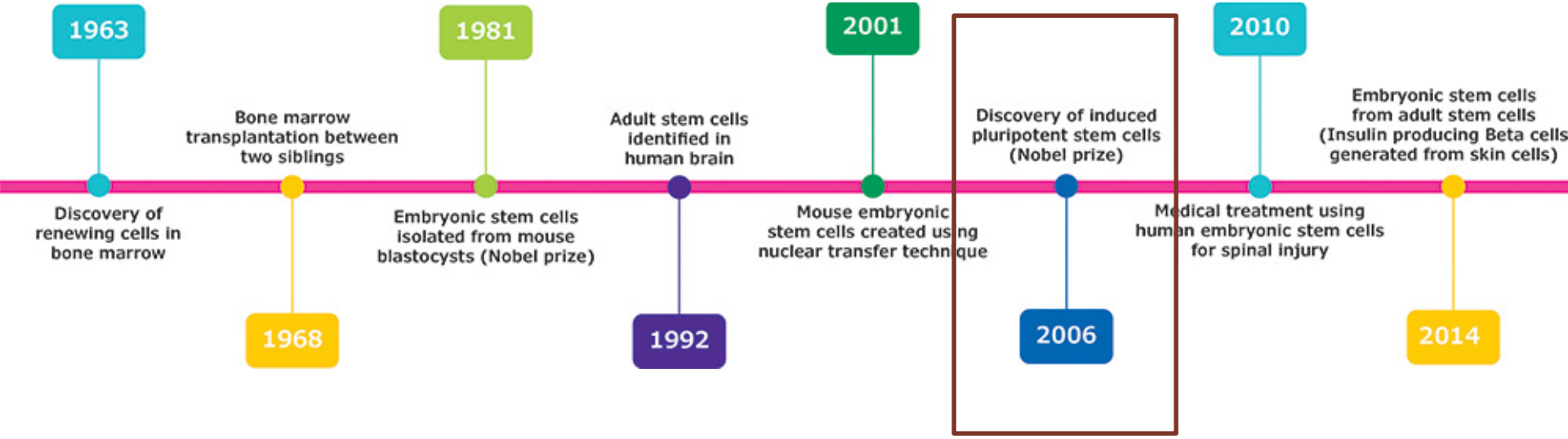
from autaptic neuronal cultures to disease modeling for neurodegeneration: recent advances in stem cell research

technical journal club

merve avar

9.7.19

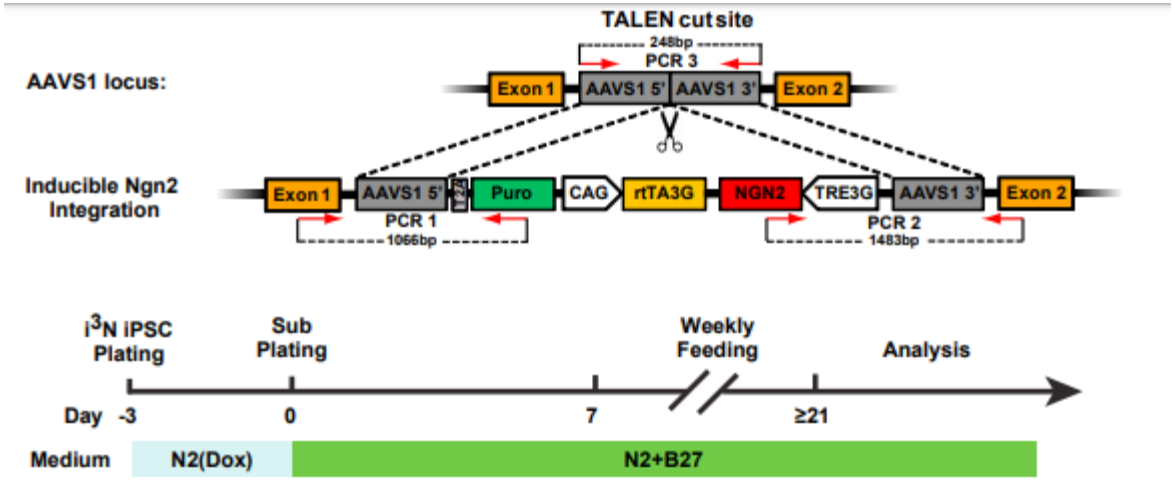
why iPSC derived neurons?



- initially cell lines used were all based on cancer cells or imortalization through a forced viral integration → relevance of such cells are questionable
- since early 2000s we have the opportunity to induce pluripotency
- derivation protocols have been tricky in development
- based on:
  - chemical
  - forced (transcription factors)
  - transposon/viral
  - miRNA initiated

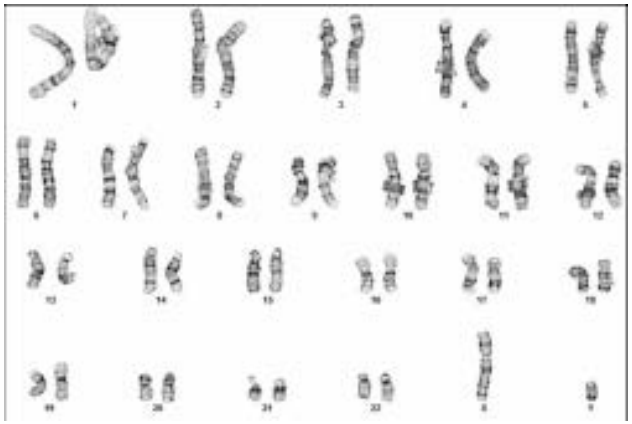
Scalable Production of iPSC-Derived Human Neurons to Identify Tau-Lowering Compounds by High-Content Screening

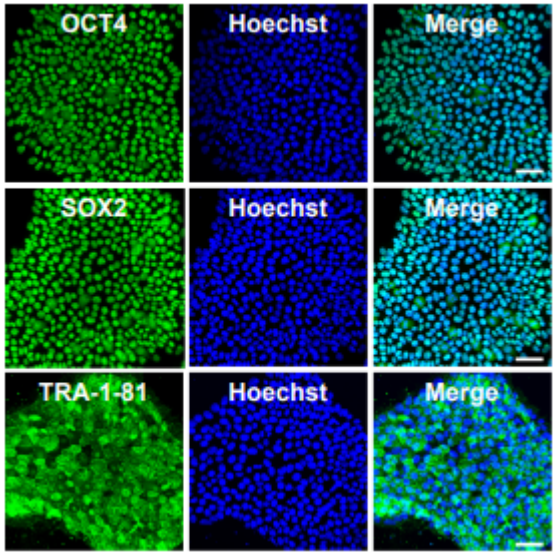
- establishment of a user friendly iPSC derivation protocol
- scaling up to 384-well format
- high content screen for tau lowering compounds
- validation of the screen

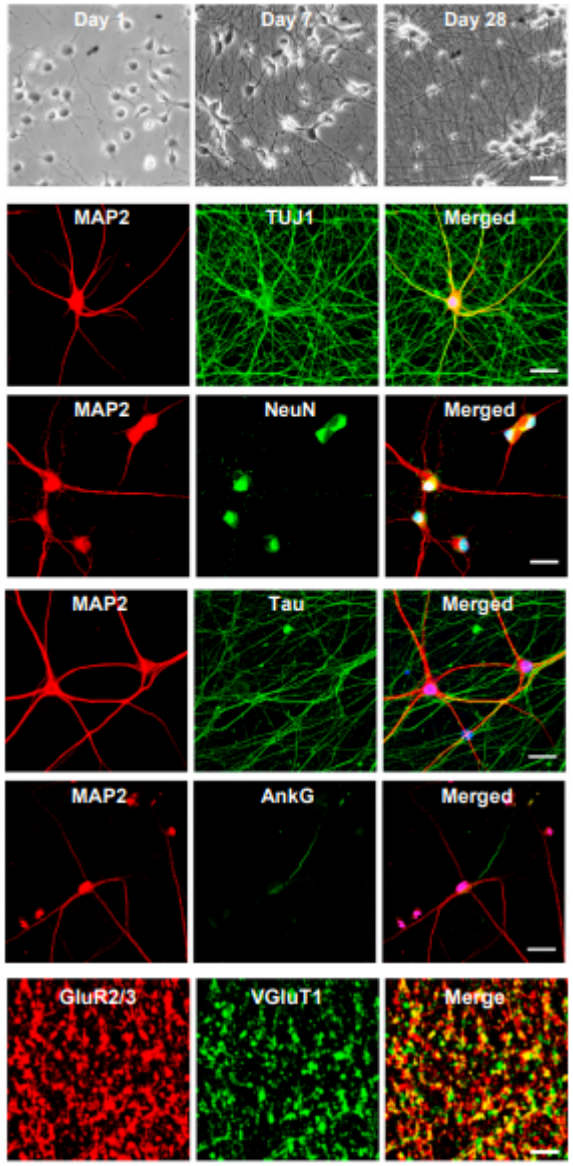


iPSC line: WTC11

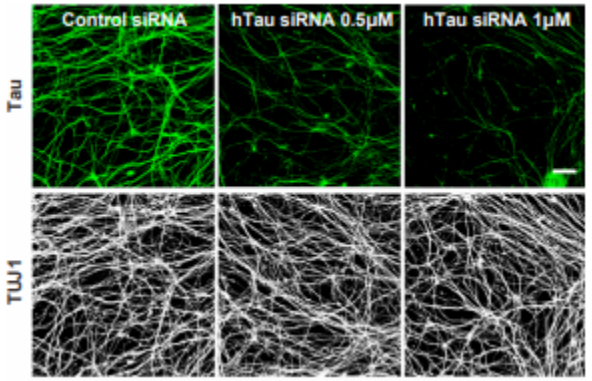
characterization and QC of i3 neurons

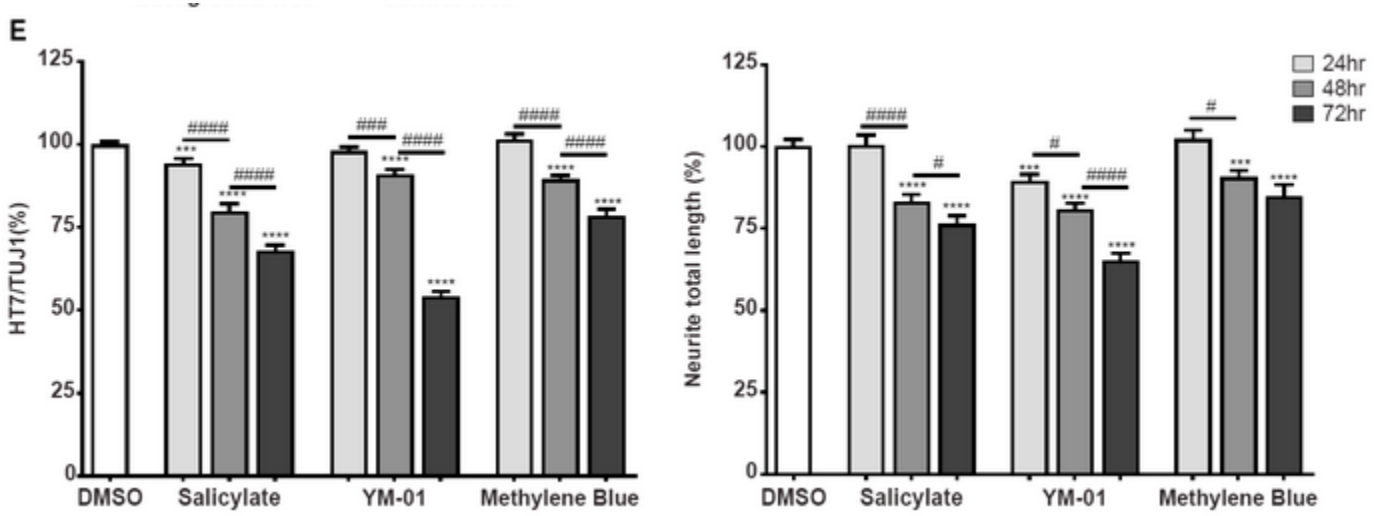






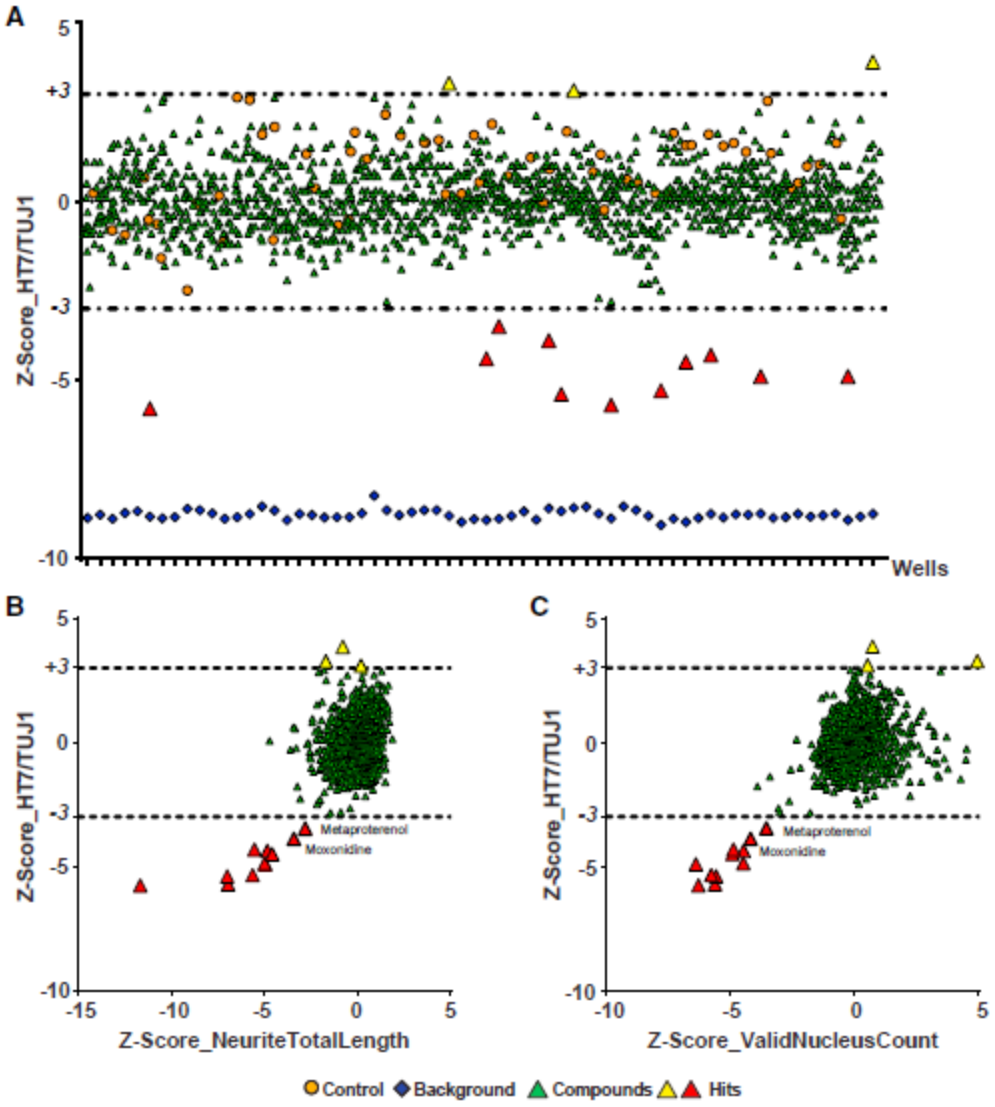
establishment of a high content screen for tau lowering compounds





- known compounds to reduce tau levels as controls as well as MAPT siRNAs
- z`factor for the screen: 0.41

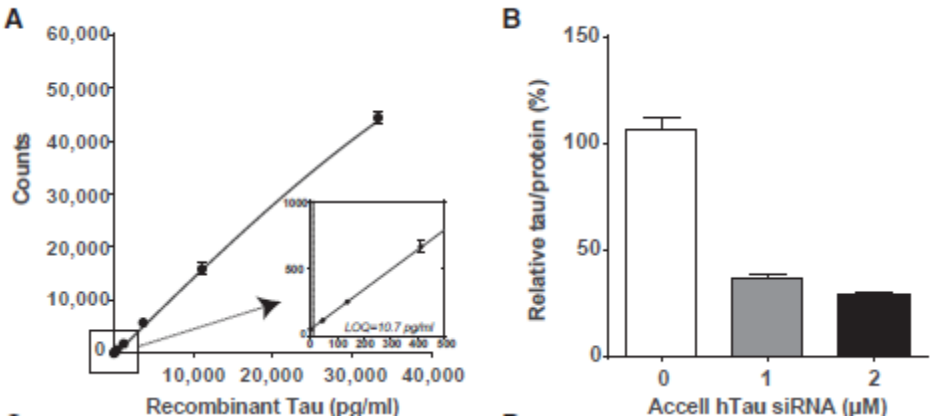
HCS results for tau lowering compounds

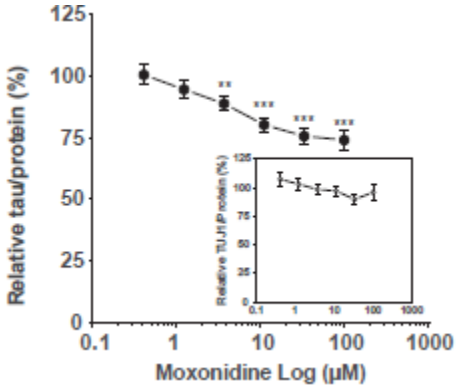


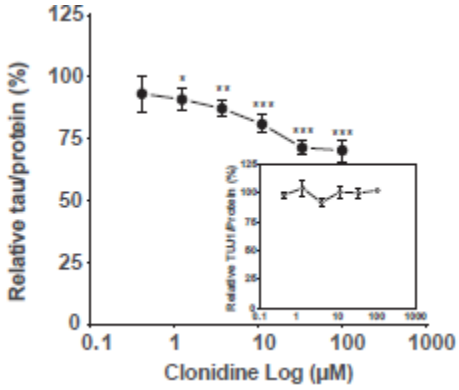
- compound library: LOPAC (library of pharmacologically active compounds)
- 1280 small molecules
- Hit calling criteria: based on z`factor of all samples per plate vs the target molecule , cutoff: -3/3 or p value of 0.00135

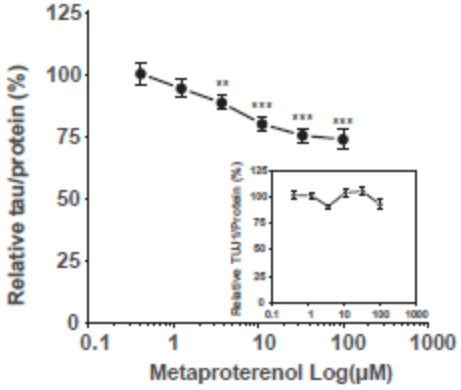
validation of the screen

- hTau ELISA (using HT7/Tau5)





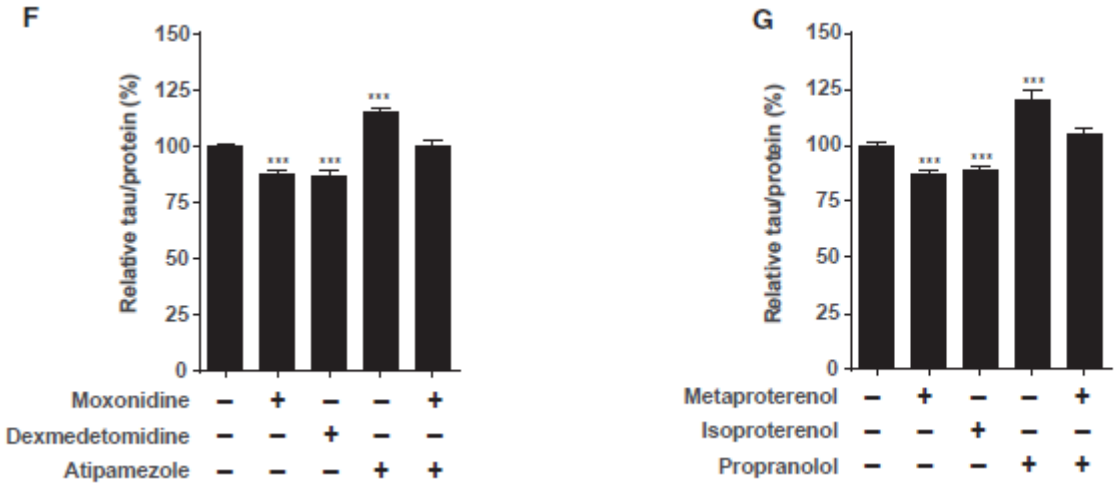




another AR agonist

validation of the screen

- hTau ELISA (using HT7/Tau5)



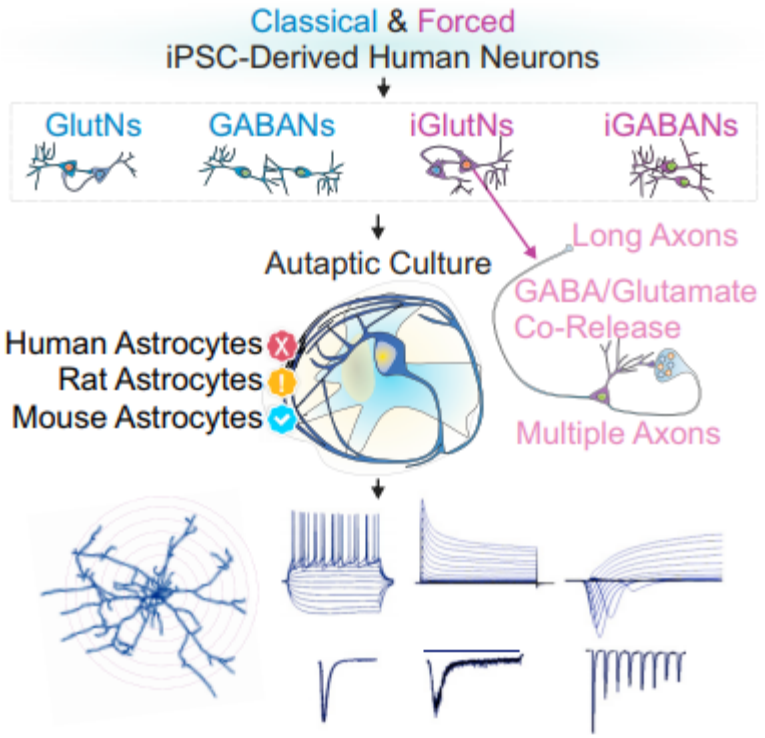
- Moxonidine, dexmedetomidine, metaproterenol: aAR agonist
- Isoproteranol: bAR agonist
- Atipamezole: aAR antagonist
- Propranolol: bAR antagonist

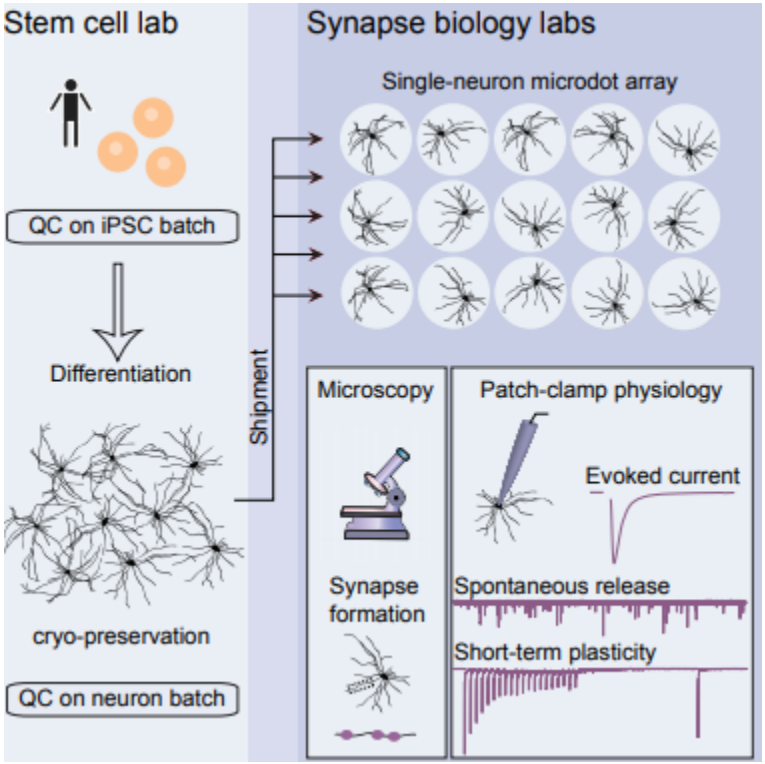
conclusions paper 1

- NGN2 insertion allows for easy to culture derivation of human glutamergic neurons
- first human neurons applicable to scaling in 384 well format → possible to adapt human neurons in a screening setup
- NGN2 neurons are available in house in case anyone would like to use them!

Cell Reports

An Autaptic Culture System for Standardized Analyses of iPSC-Derived Human Neurons



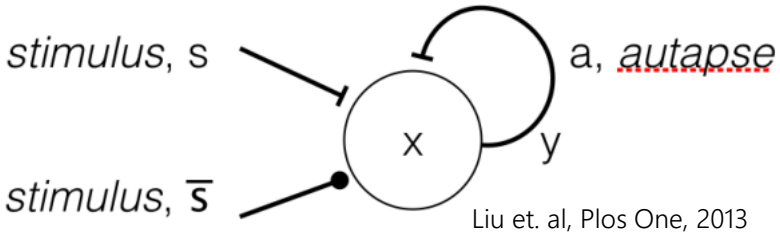


Cell Reports

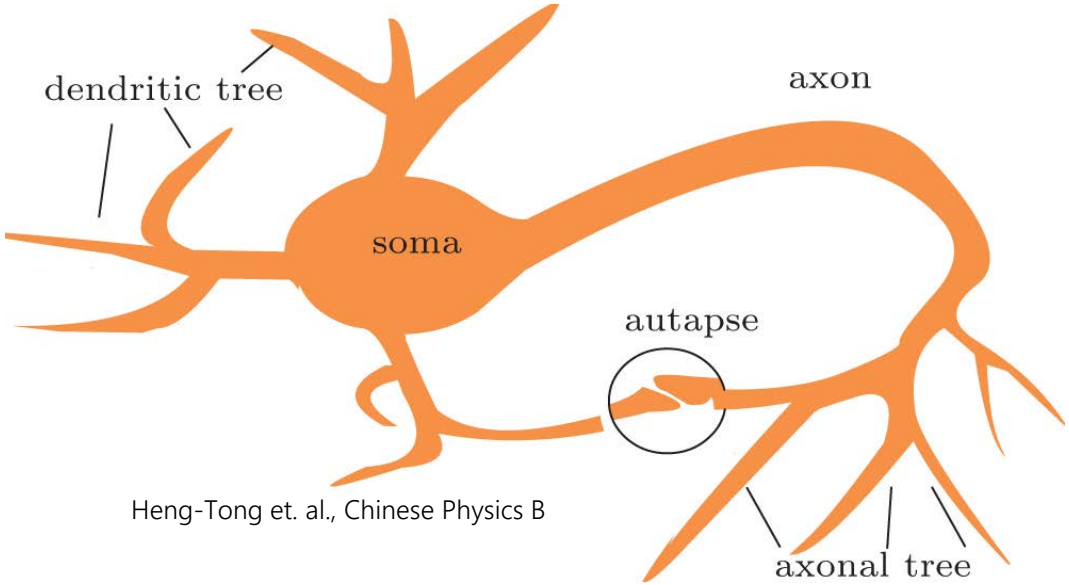
A Single-Cell Model for Synaptic Transmission and Plasticity in Human iPSC-Derived Neurons

autapses

- autapse: synapse of a neuron onto itself
- naturally occuring in the brain especially in the neocortex: inhibitory fast-spiking cells have abundant autapses
- autaptic self- inhibiton is thought to be important in maintaining firing precision of a neuron thereby contributing to the pacing of neuronal networks
- good model to study synapses



Liu et. al, Plos One, 2013



Heng-Tong et. al., Chinese Physics B

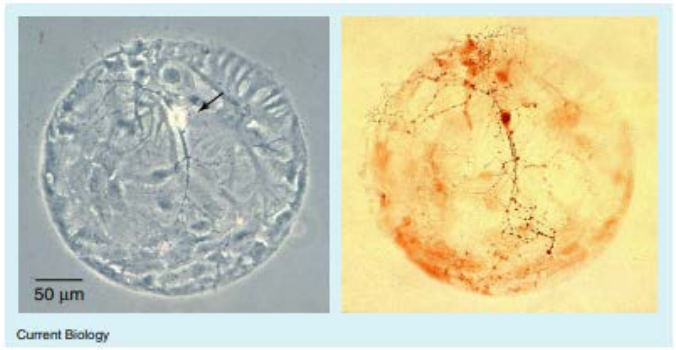


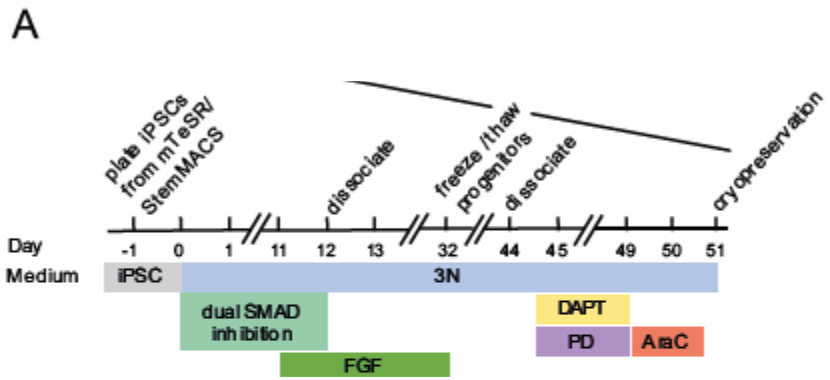
Figure 1. A single rat hippocampal neuron (arrowed, left) grown in culture on a 'microdot' of glia (flat gray cells), showing abundant autapses labeled with an antibody (small dark spots, right).

Ikeda, Cell, 2006

overview

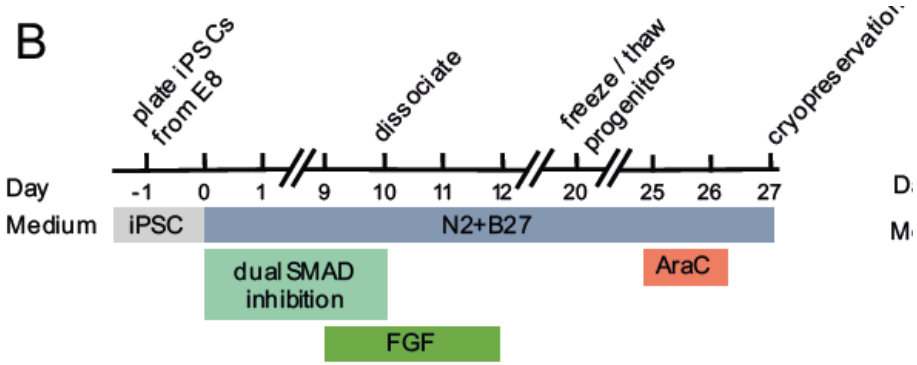
- two ways of deriving neuronal cells: classical vs transcription factor mediated
- FBS content
- seeding count for microislands
- characterization and QC
- mouse/human/rat astrocyte coculture comparison
- synapse characterization

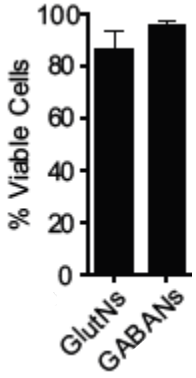
generation of neurons – classical derivation



Glutamergic neurons (GlutN)

dual SMAD inhibitors (conversion into neuroectoderm)
supplementation of FGF
DAPT (Notch/gamma secretase inhibitor)
PD0325901 (MEK/ERK inhibitor)
AraC (for postmitotic state promotion)

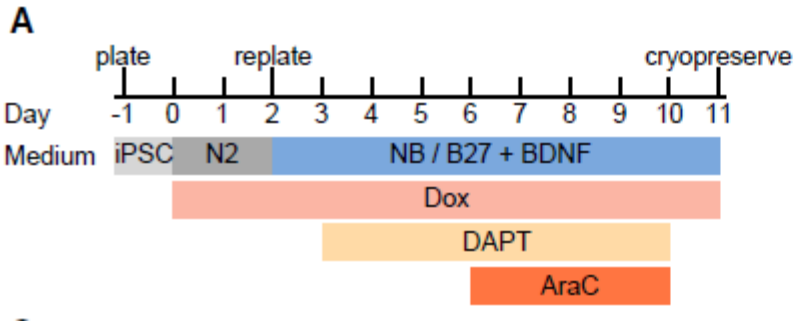


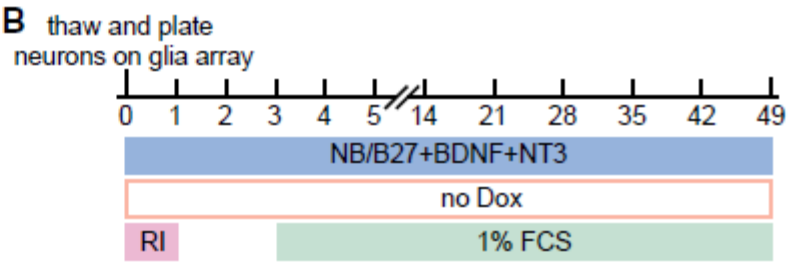


dual SMAD inhibitors (conversion into neuroectoderm)
supplementation of FGF
AraC (for postmitotic state promotion)

GABAergic neurons (GABAN)

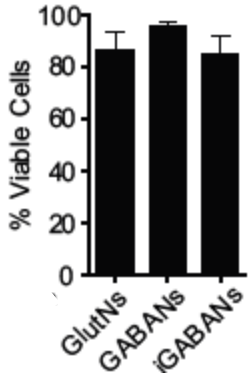
generation of neurons – TF mediated

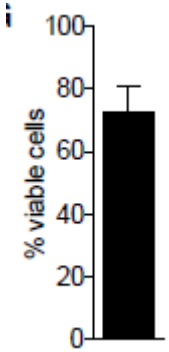


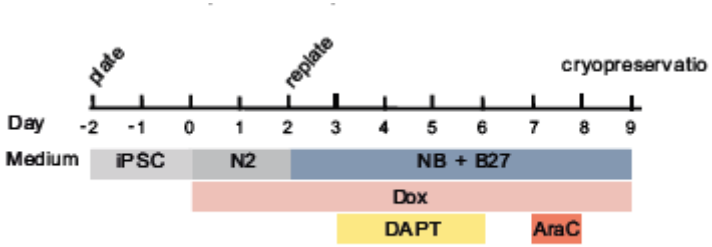


Doxycyline induction
DAPT (Notch/gamma secretase inhibitor)
AraC (for postmitotic state promotion)

Glutamergic neurons (iGlutN)
NGN2



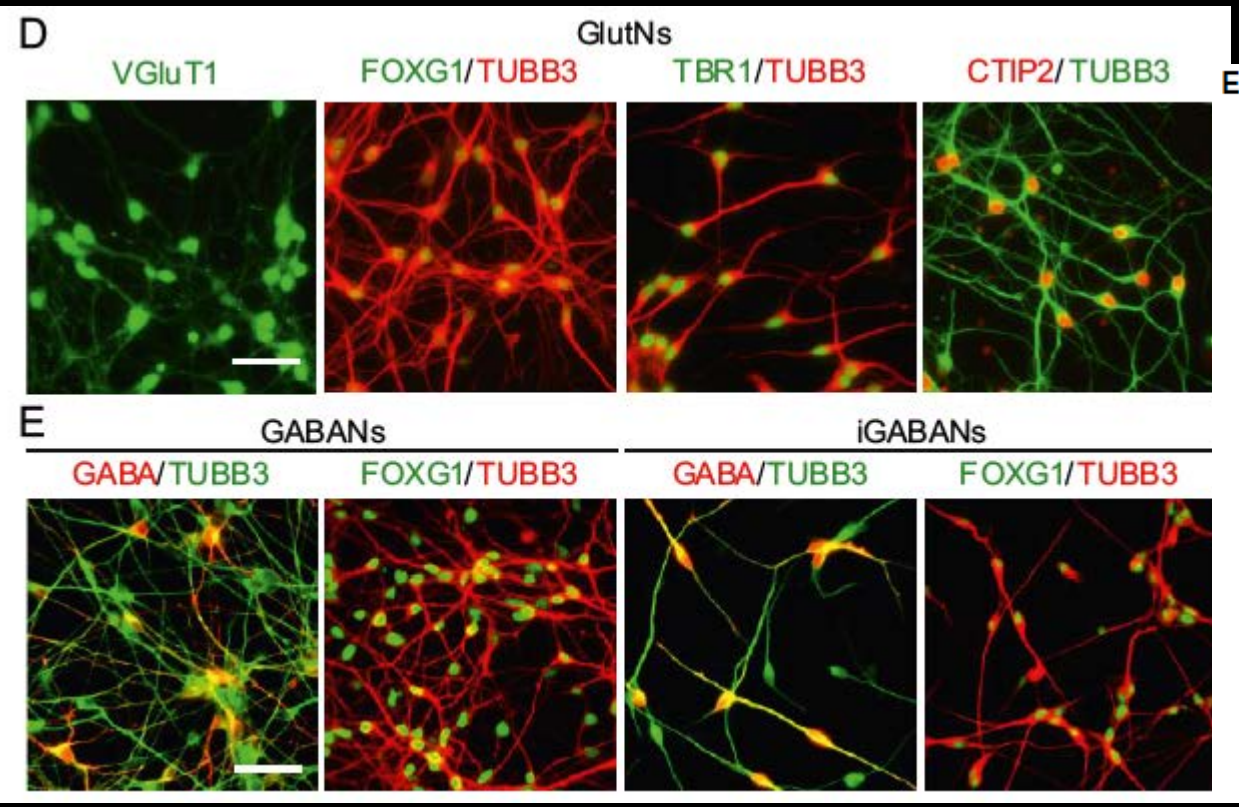




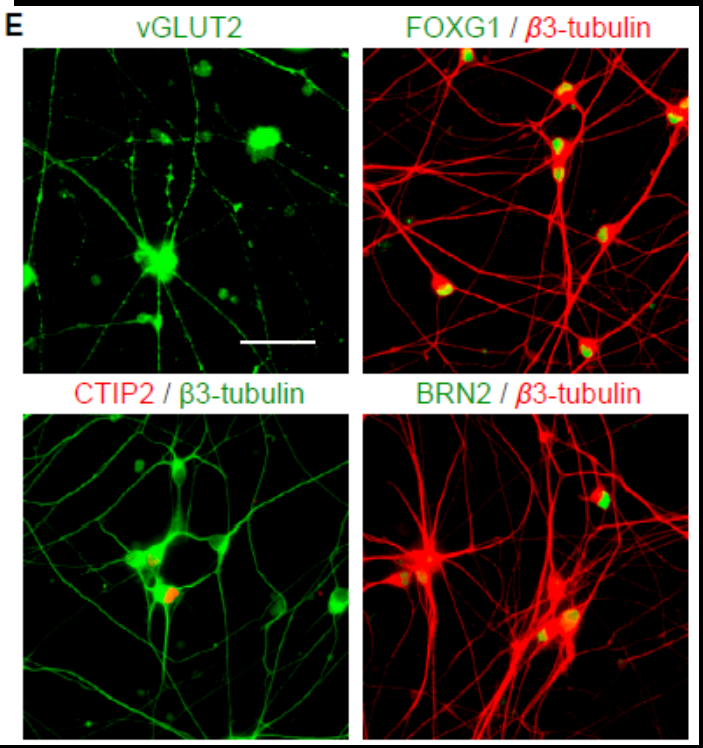
Doxycyline induction
DAPT (Notch/gamma secretase inhibitor)
AraC (for postmitotic state promotion)

GABAergic neurons (iGABAN)
DLX2 and ASCL1

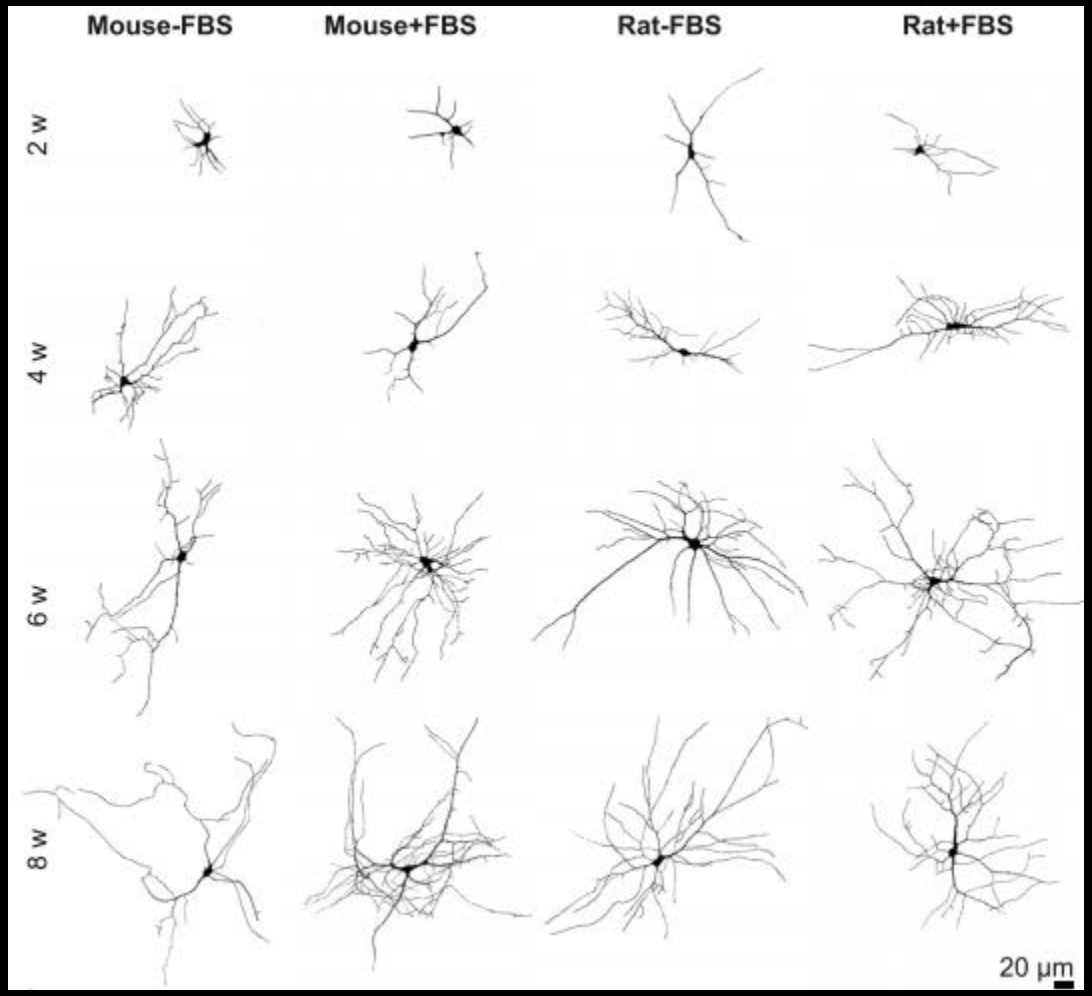
characterization and QC GlutN / GABAN / iGluTN / iGABAN cultures

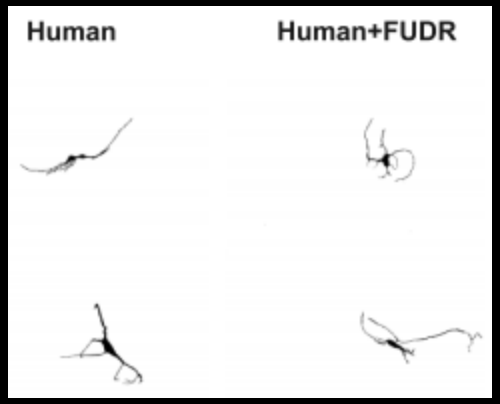


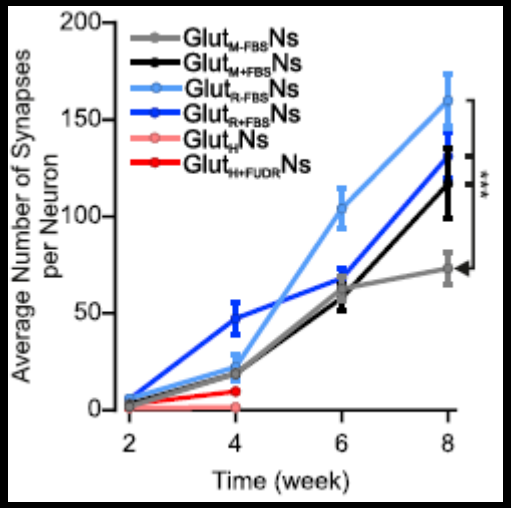
iGlutN



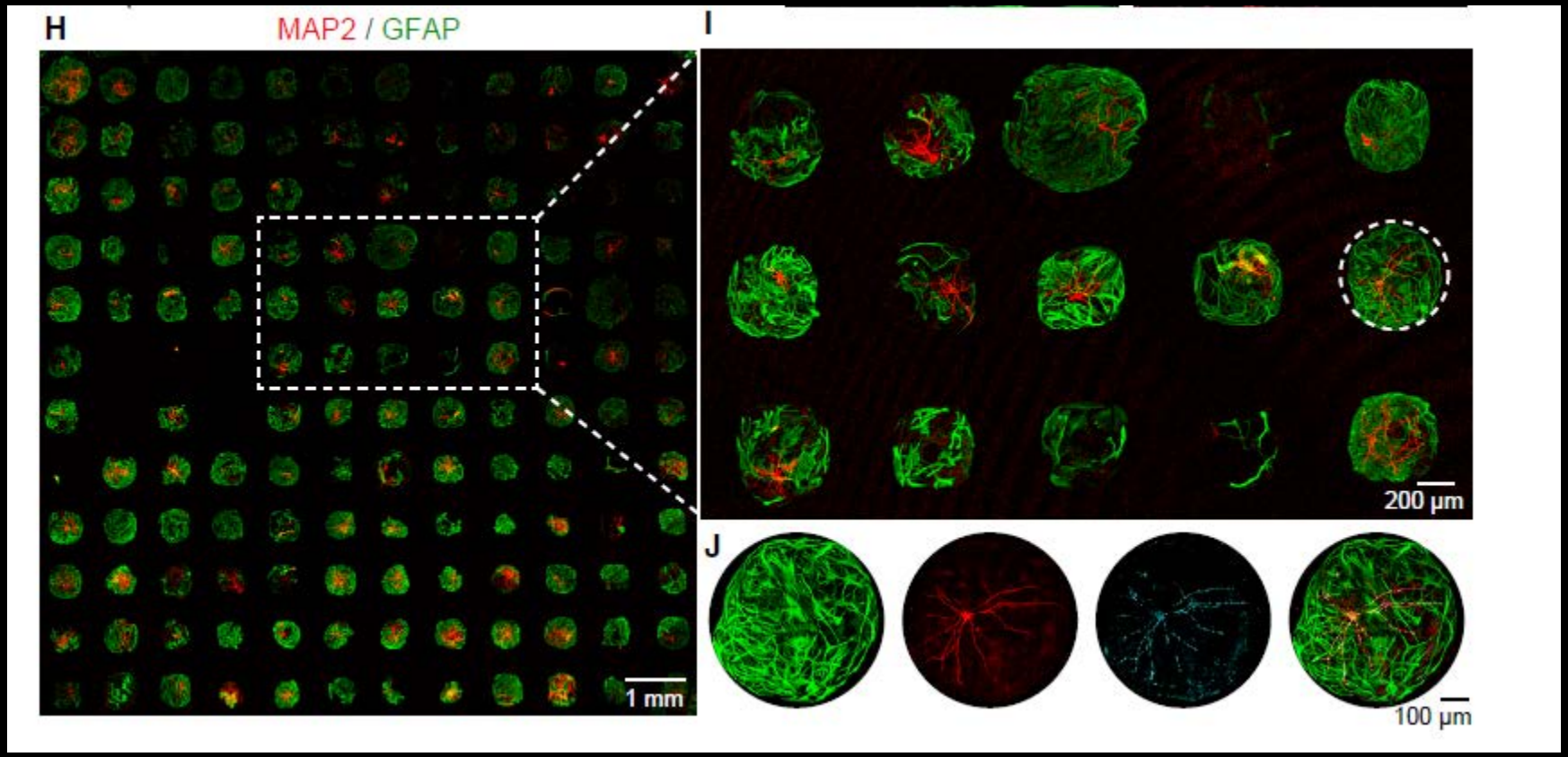
Testing rat/mouse and human astrocytes for support of autaptic GlutN cultures



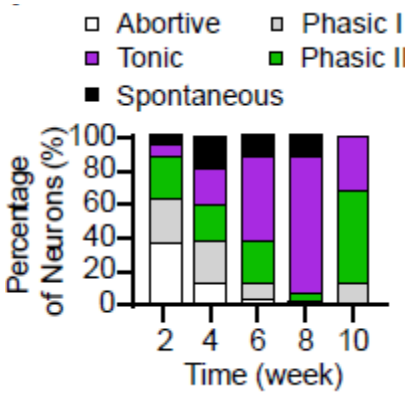


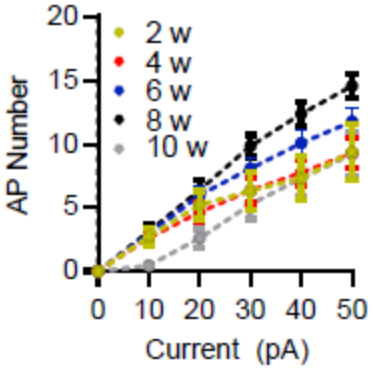


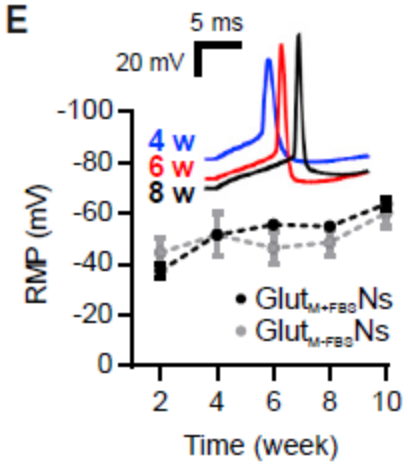
iGLutNs autaptic cultures on microdot arrays

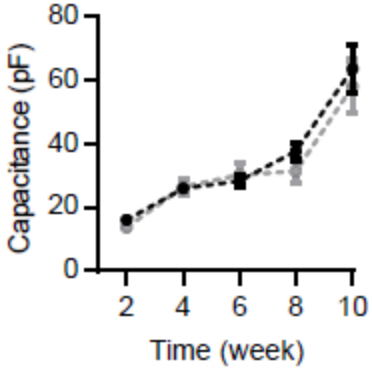


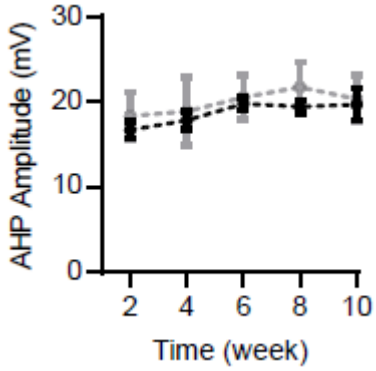
Membrane properties of GluTNs

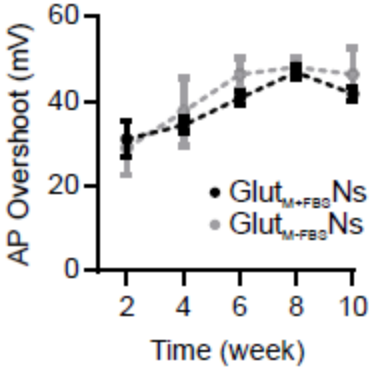












conclusions paper 2

- Chemical derivation is cumbersome, however generation of pure cultures are possible (GlutN and GABAN production)
- iGABAN and iGlutN cultures are based on transcription factor mediaed differentiation and even shortens the initial reportings in neuronal maturation time
- autaptic cultures are single neurons grown on glia islands and allow for study of synapses, making it possible to spike AP in a cell and simultaneous recording in the same cell

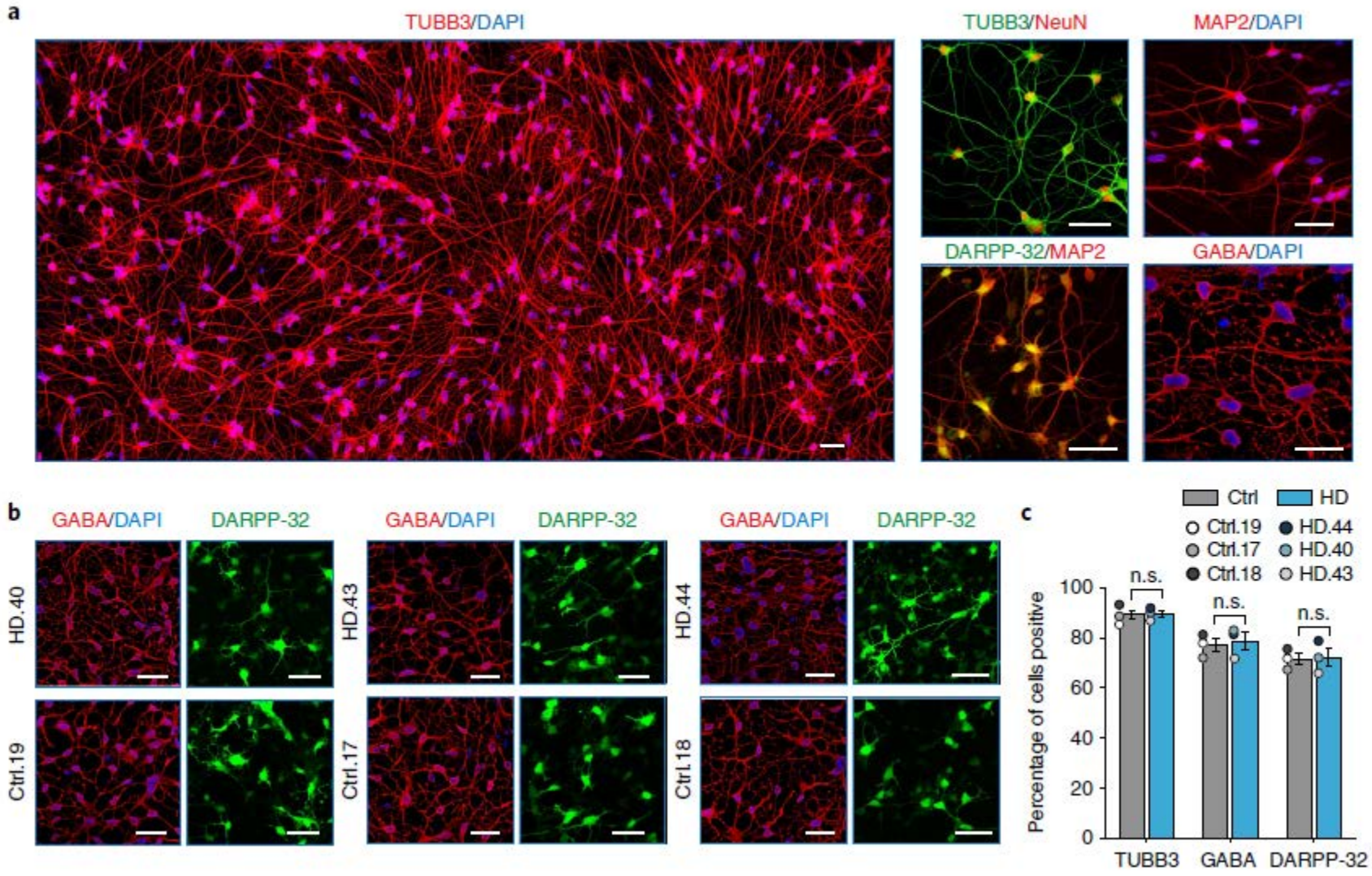
Striatal neurons directly converted from Huntington's disease patient fibroblasts recapitulate age-associated disease phenotypes

thus far, there have not been any relevant models in iPSC derived neurons for any neurodegenerative disease

open question: can age signature be indispensable in recapitulating disease?

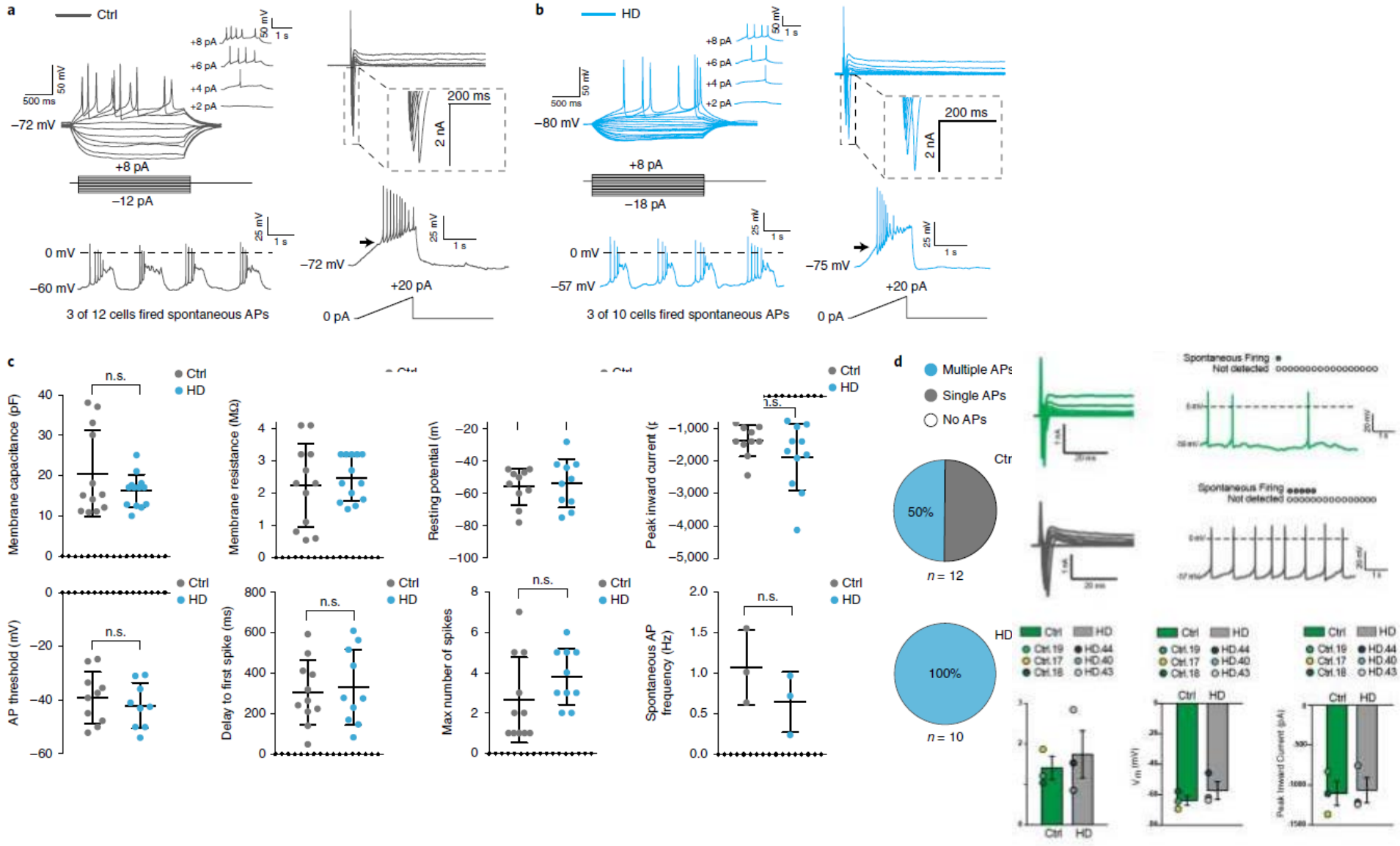
- miRNA based direct conversion from Huntington`s patients and healthy donors
- characterization of the model
- phenotypical findings

generation of MSNs from HD patient fibroblasts – miRNA based derivation

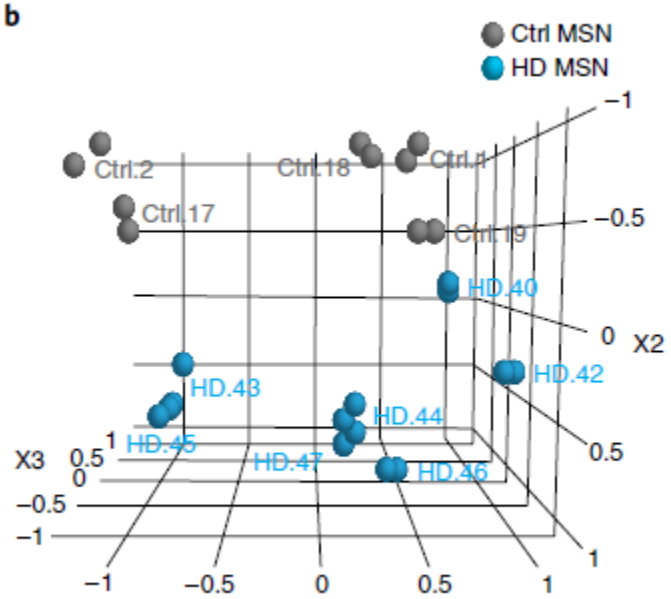


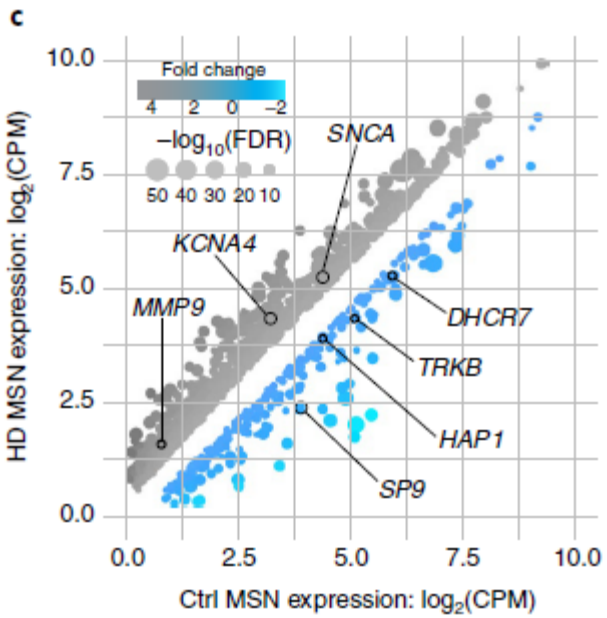
Day 30 post induction, 70-80% GABAergic neurons
CAG repeats <50, reflects most HD cases, they remain stable after neuronal conversion

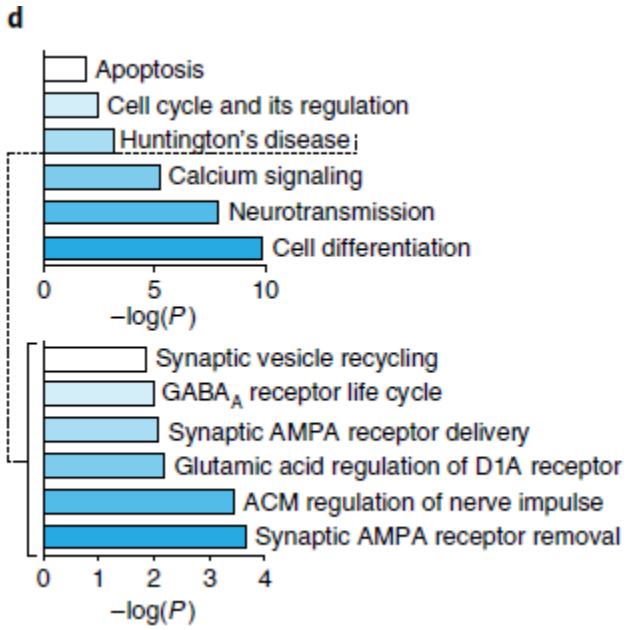
derivation of MSNs from patient and healthy donor fibroblasts



RNA-Seq of patient vs control neurons

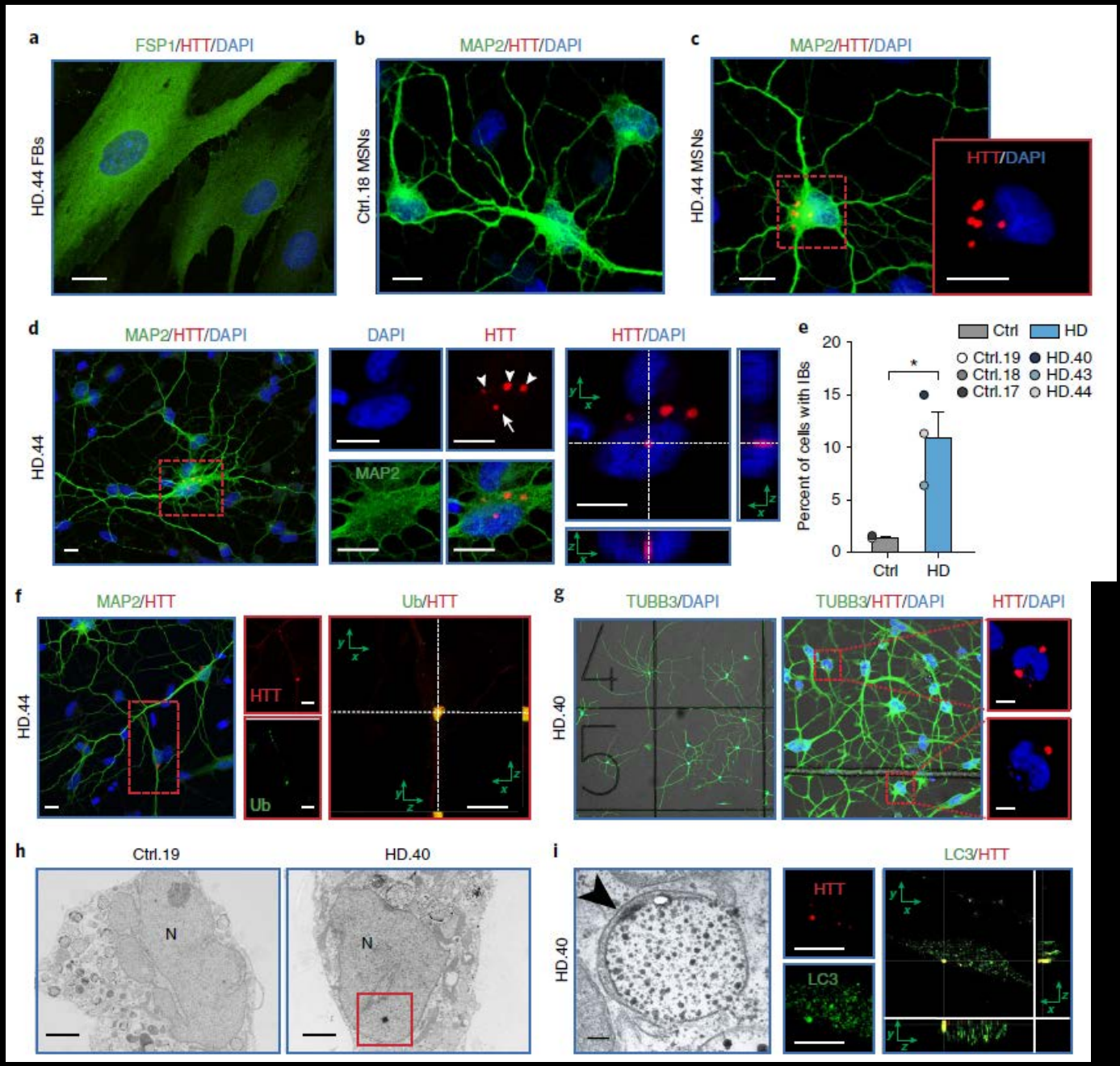






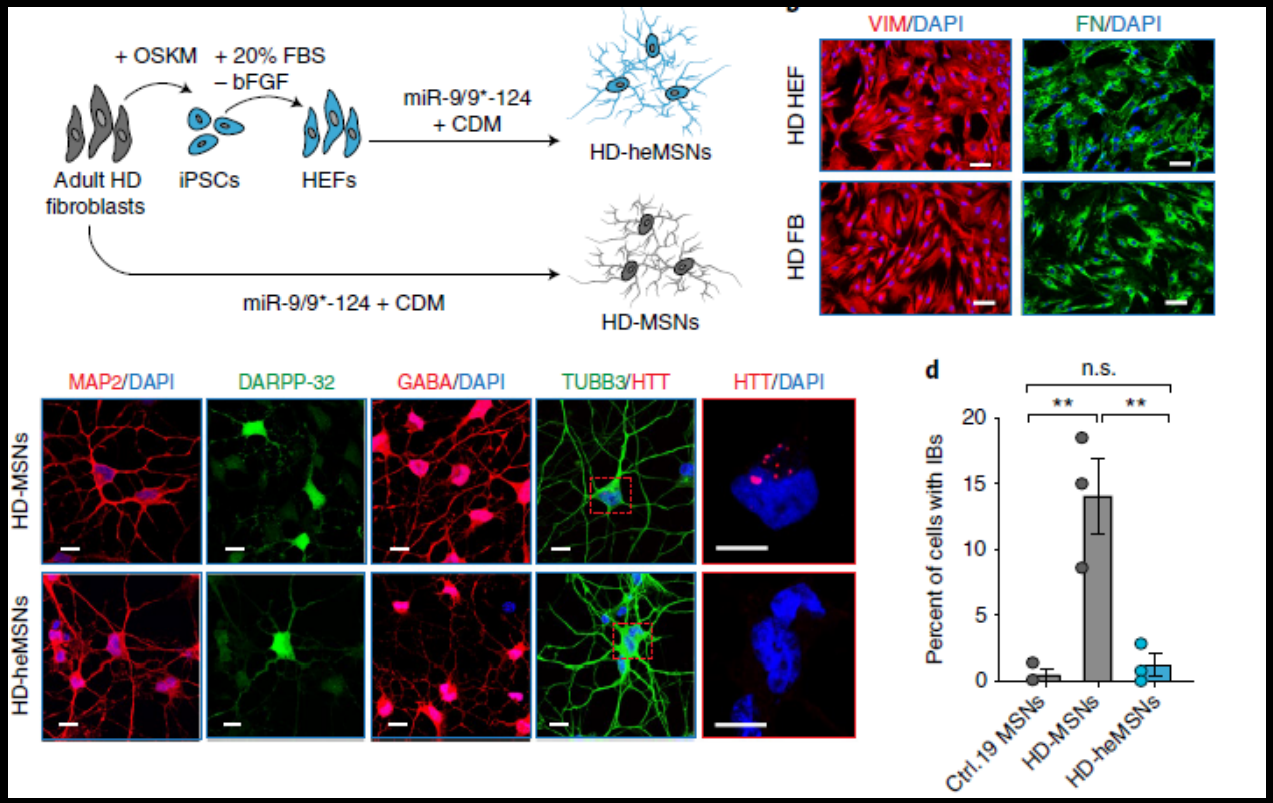
32d post induction
7 HD patients and 5 controls

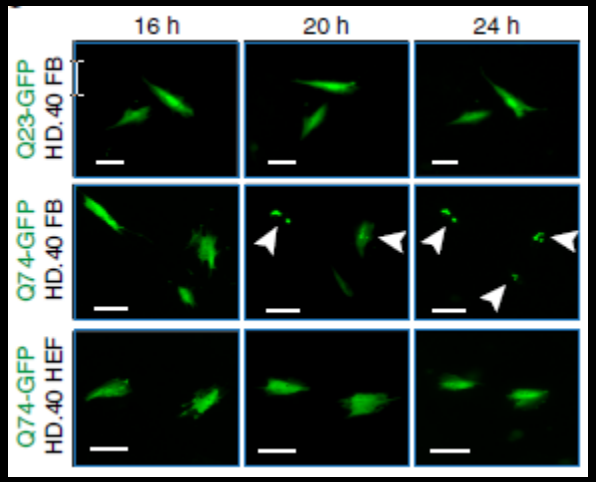
aggregation of mHTT in patient derived MSNs

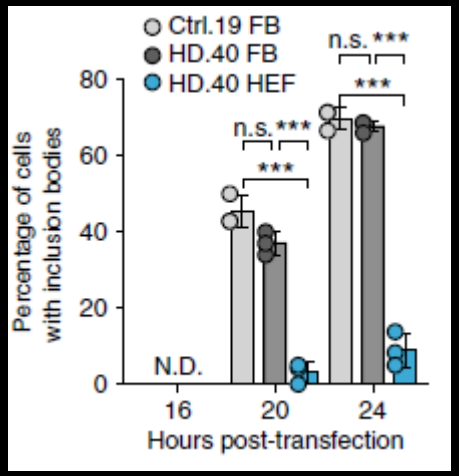


30d post induction

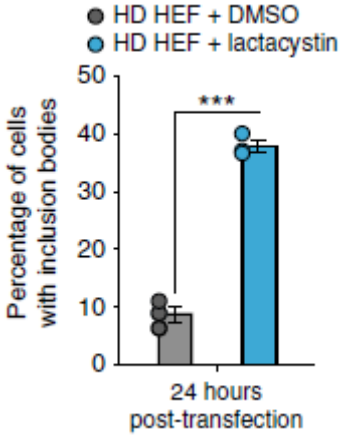
Comparison of derivation of MSNs from iPSCs

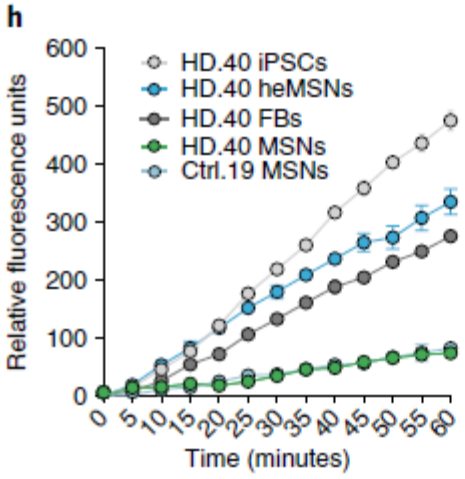


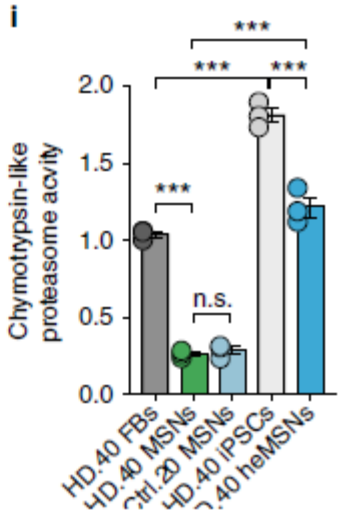


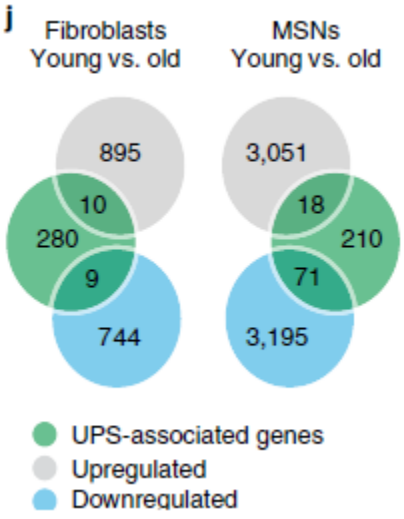


proteostasis in HD patient derived MSNs

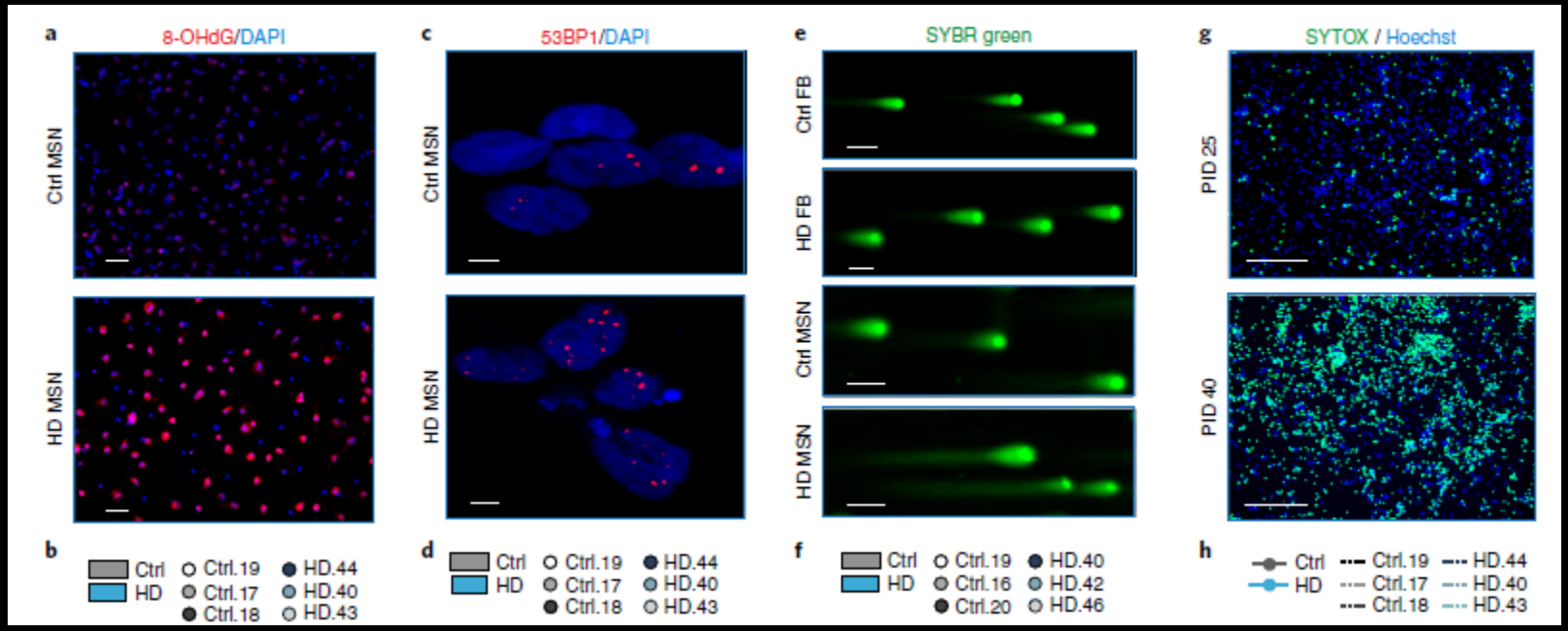


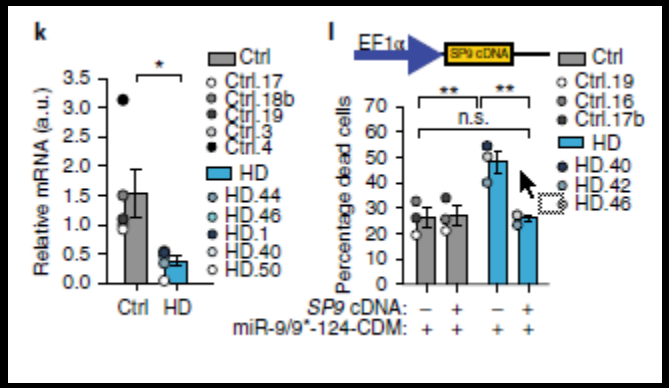


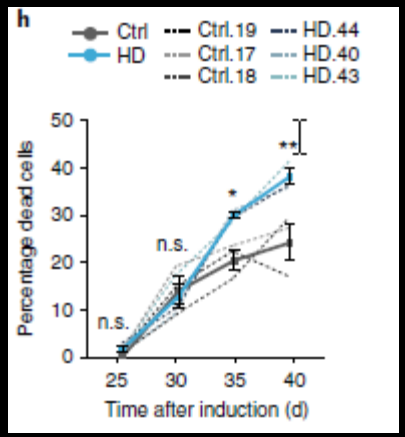




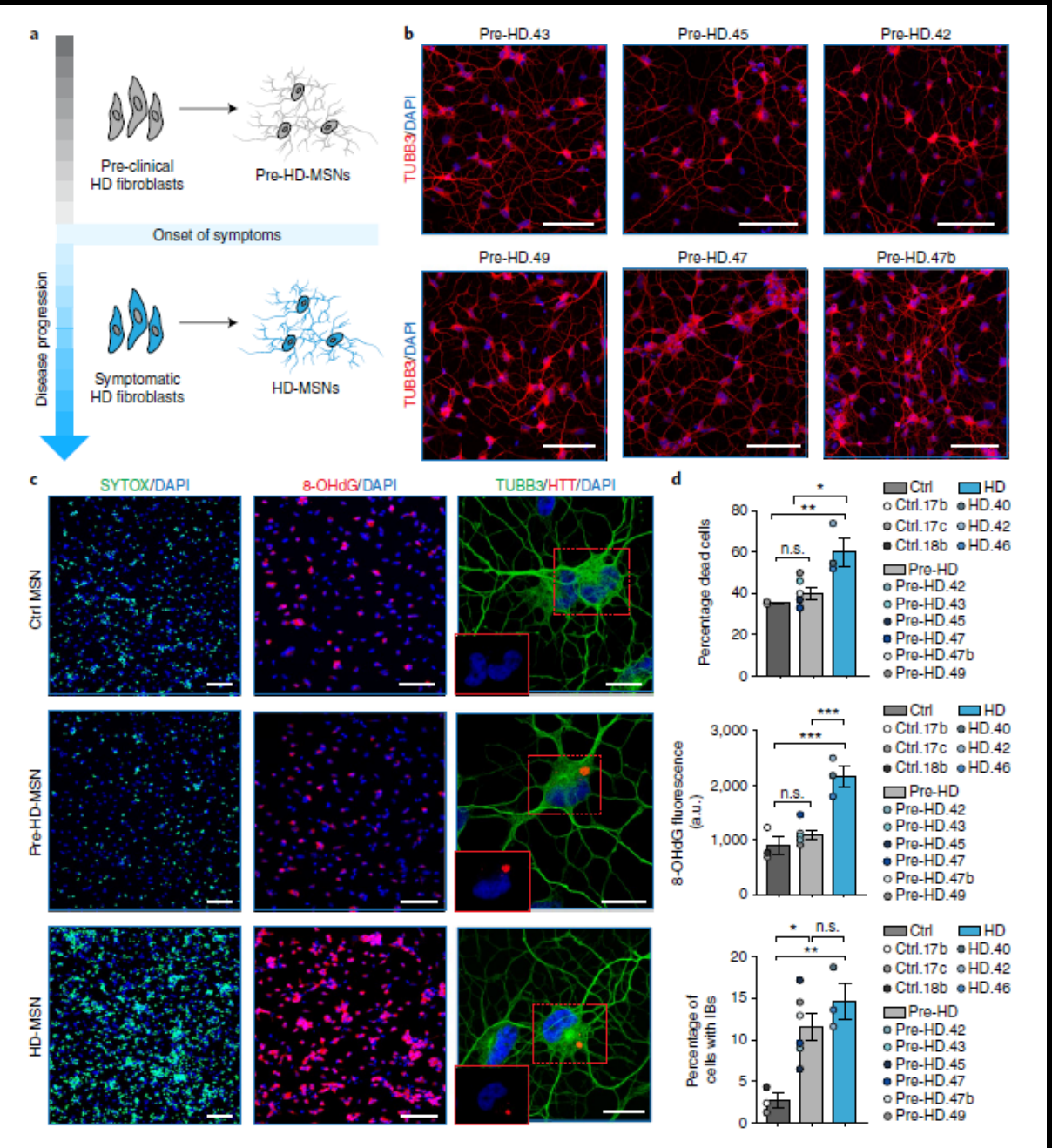
DNA damage and spontaneous degeneration in HD patient derived MSNs







HD samples reprogrammed from presymptomatic patients



conclusions paper 3

- first reporting of a neurodegenerative disease model recapitulating pathology without introduction of additional insults
- age signature is indispensable
- HD MSNs show accumulation of mHTT, DNA damage, mitochondrial damage as seen in HD patients
- neurons generated from fibroblasts of HD patients go through degeneration in culture from 35d post induction