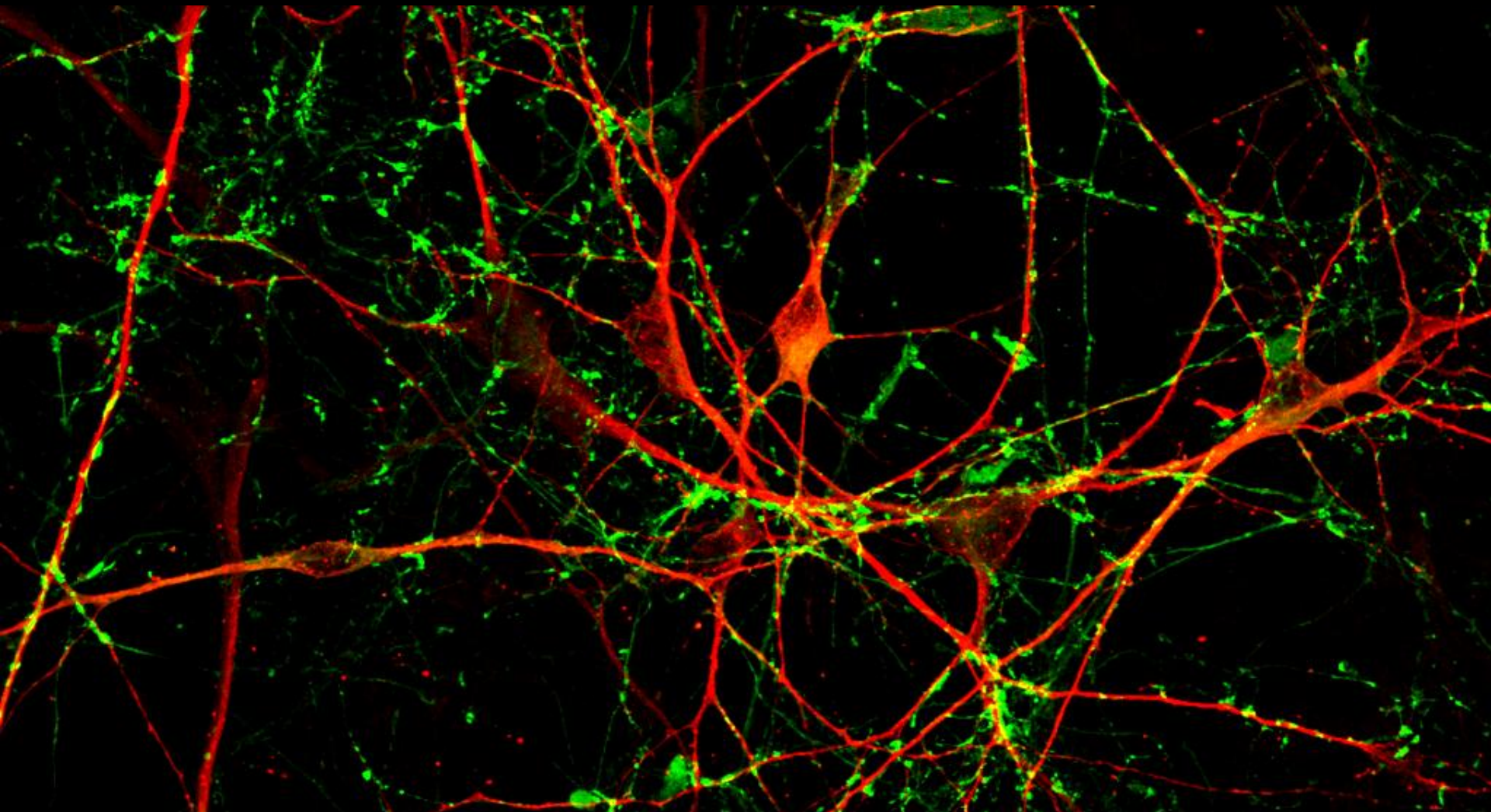
Investigating familial Parkinson's disease using a stem cell model



6M PARKINSON'S DISEASE PATIENTS WORLDWIDE

1M PATIENTS IN EUROPE

€13.9 BILLION ANNUAL EUROPEAN COST

BUT... **NO CURE**, YET

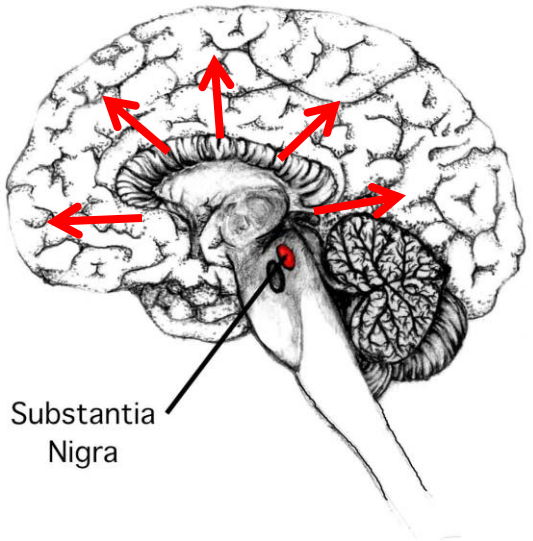
Both environmental and genetic factors contribute to Parkinson's disease risk

Most cases are late-onset idiopathic forms

Rare familial forms: a number of mutations have been identified in several genes that are linked to autosomal dominant or recessive familial forms of the disease: LRRK2, PINK1, alpha-synuclein SNCA (point mutations, duplications, triplications)

Parkinson's Disease

James Parkinson 'An Essay on the Shaking Palsy', 1817



Motor symptoms

resting tremor, rigidity, bradykinesia and postural instability

Non-motor symptoms

cognitive and sensory disturbances

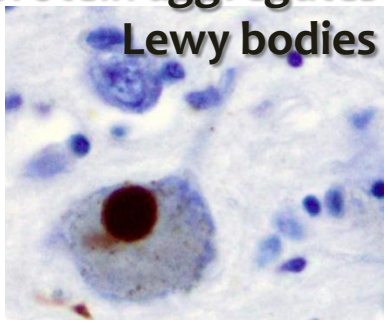
Parkinson's Disease

αSynuclein gene

G209A (**p.A53T**) αSyn mutation causes a **familial form of PD** characterized by **early onset** and a generally severe phenotype, including **non-motor manifestations** (Polymeropoulos et al, Science 1997)

alpha-synuclein protein aggregates

Lewy bodies



Sir John Gurdon Shinya Yamanaka

2012 Nobel Prize
in Medicine or Physiology

Parkinson's Disease Model

induced pluripotent stem cells (iPSC)

Skin cell

Human Parkinsonian Neurons

Parkinson's Disease Patient

Preclinical Studies (in vitro)

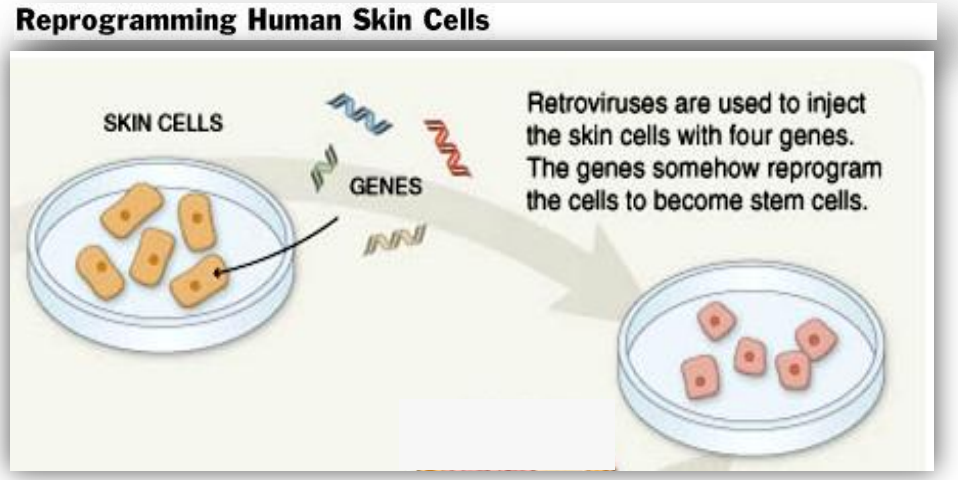
Novel drugs

Drug screening

Preclinical Studies (in vivo)

Cellular reprogramming technology:

Generation of iPS Cell Lines from patients with familial PD and healthy individuals



Oct 2011- Jan 2012

3-month Secondment in Institut Pasteur, Paris, France

Dr. Delphine Bohl, Dr Jean-Michel Heard

Retroviruses and Gene Transfer Unit

Neuroscience Department

NeuroSign

Human iPS Cell lines

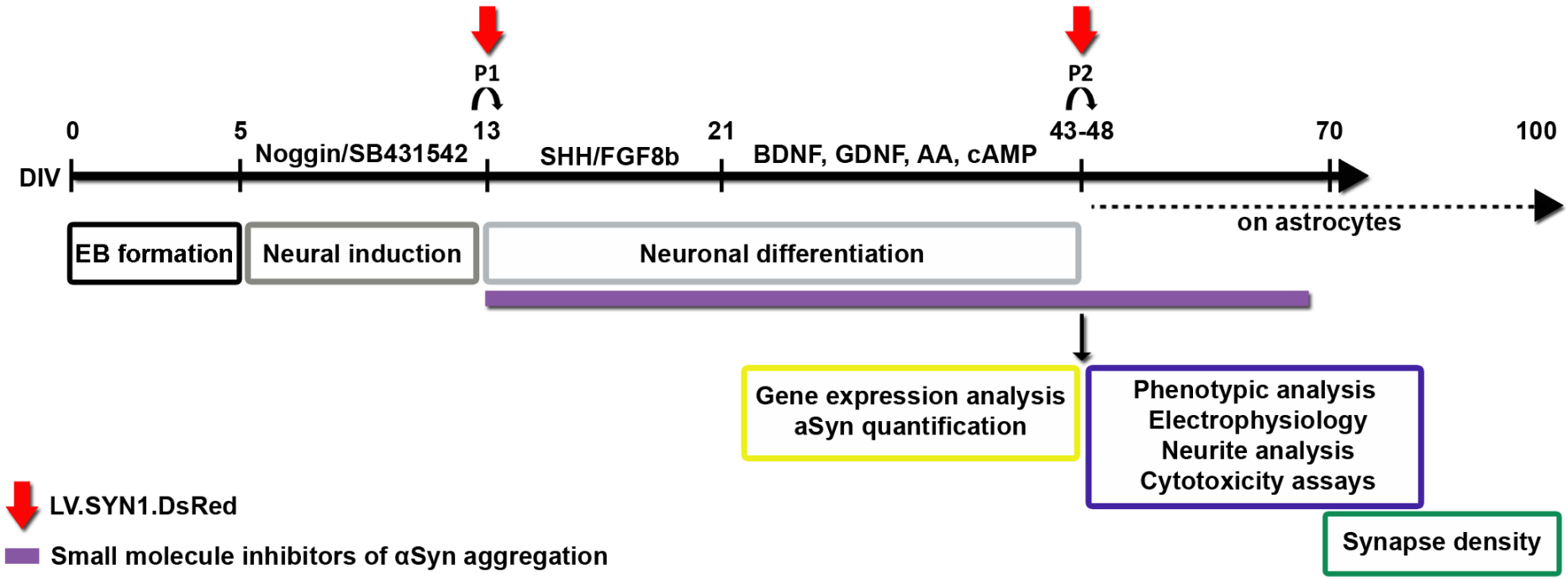
Original tissue: PD Patient-derived Skin Fibroblasts (Dr. Stefanis & Vekrelis , BRFAA)

4 Greek PD patients carrying the G209A (p.A53T) mutation in α-synuclein gene

INDIVIDUAL	AGE	SEX	LINES
Patient 1	49	male	36
Patient 2	67	female	2
Patient 3	40	male	5
Patient 4	38	female	1
Healthy 1	45	male	18
Healthy 2	45	female	14

A53T patient-specific iPSC-based model for PD

Differentiation protocol and timeline of analysis



Soldner et al, Cell 2009; Chambers et al, Nat Biotechnol 2009; Kouroupi et al, PNAS 2017 in press

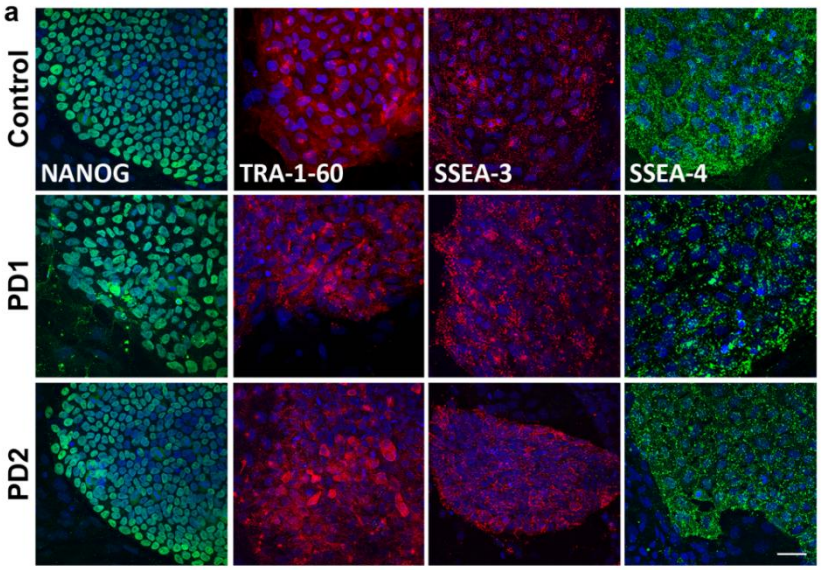
Generation and characterization of human iPSCs

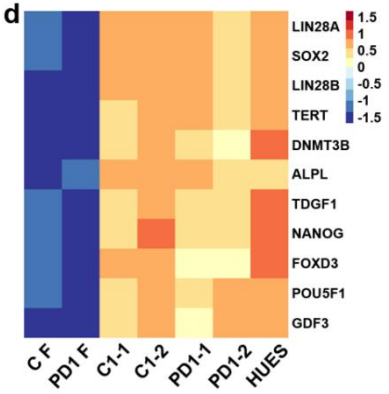
Pluripotent genes

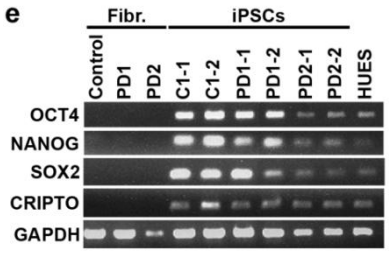
Embryoid Bodies

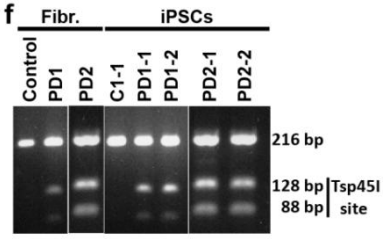
Teratoma

Karyotype (Alpha Lab)

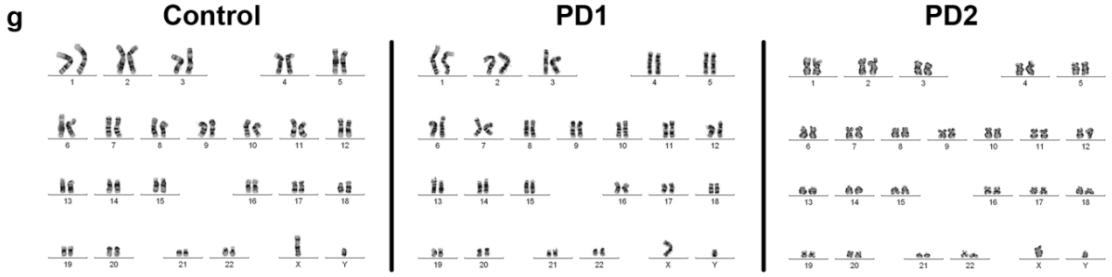




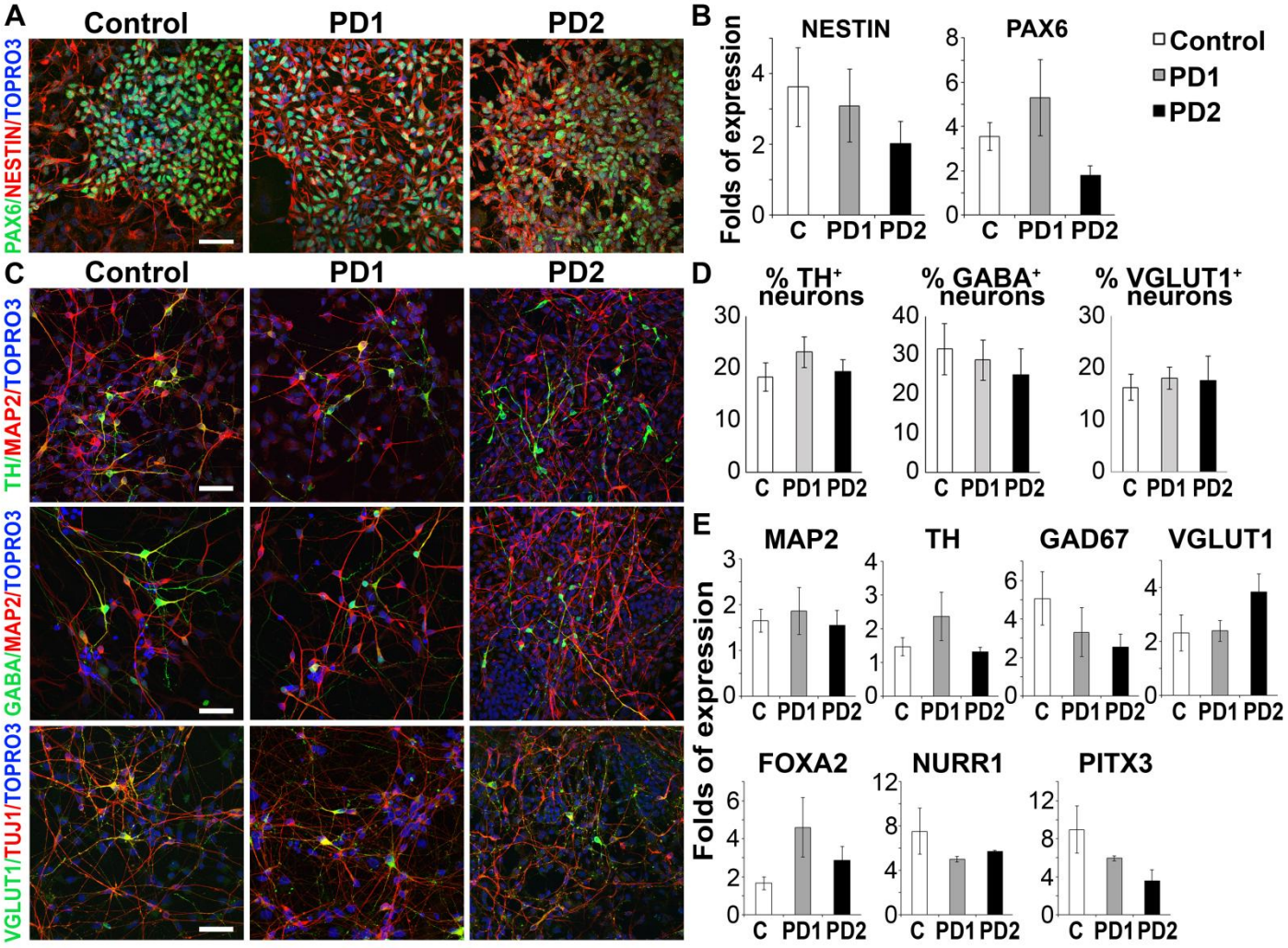




G209A SNCA mutation

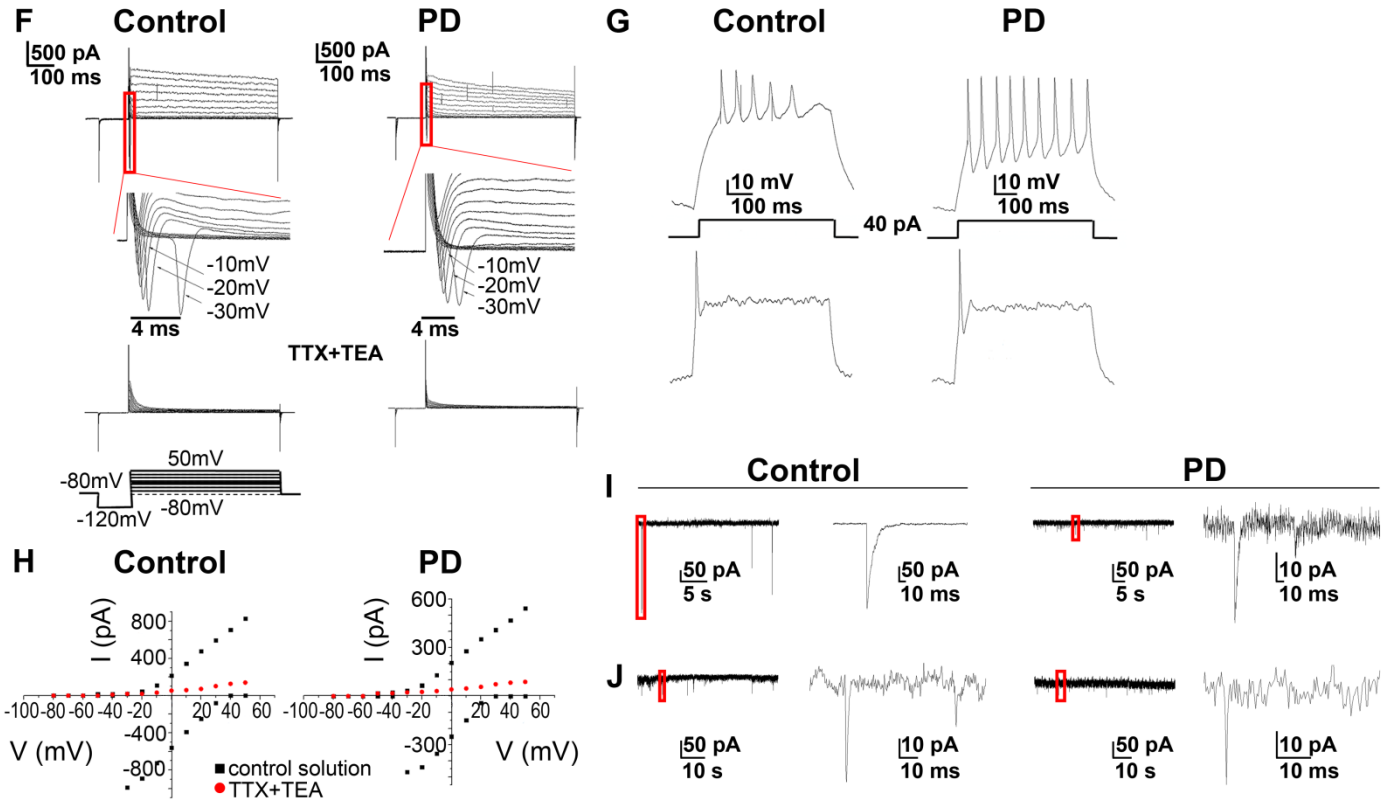


A53T patient-specific iPSC-based model for PD



A53T patient-specific iPSC-based model for PD

Electrophysiology study of iPSC-derived neurons



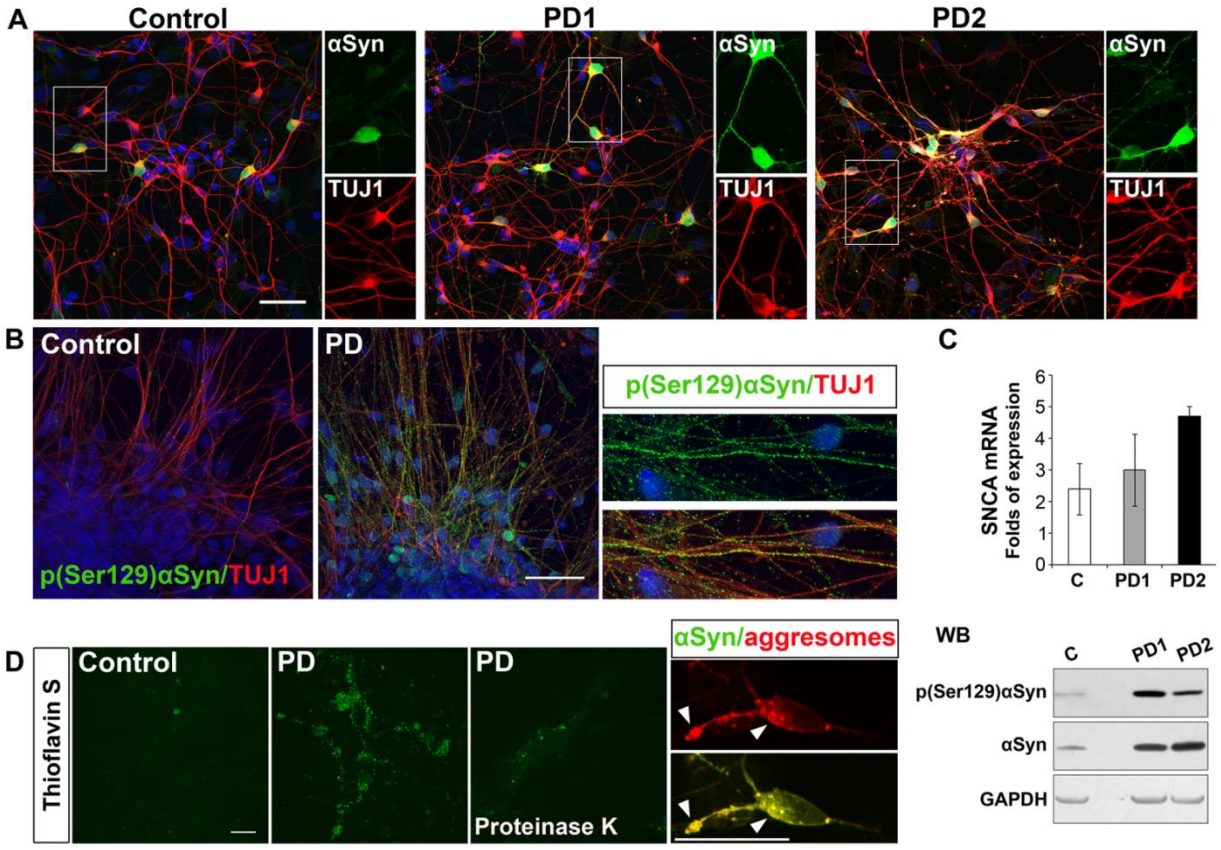
A53T-neurons displayed disease-relevant phenotypes

1. **protein aggregates, also containing αSyn**

αSyn

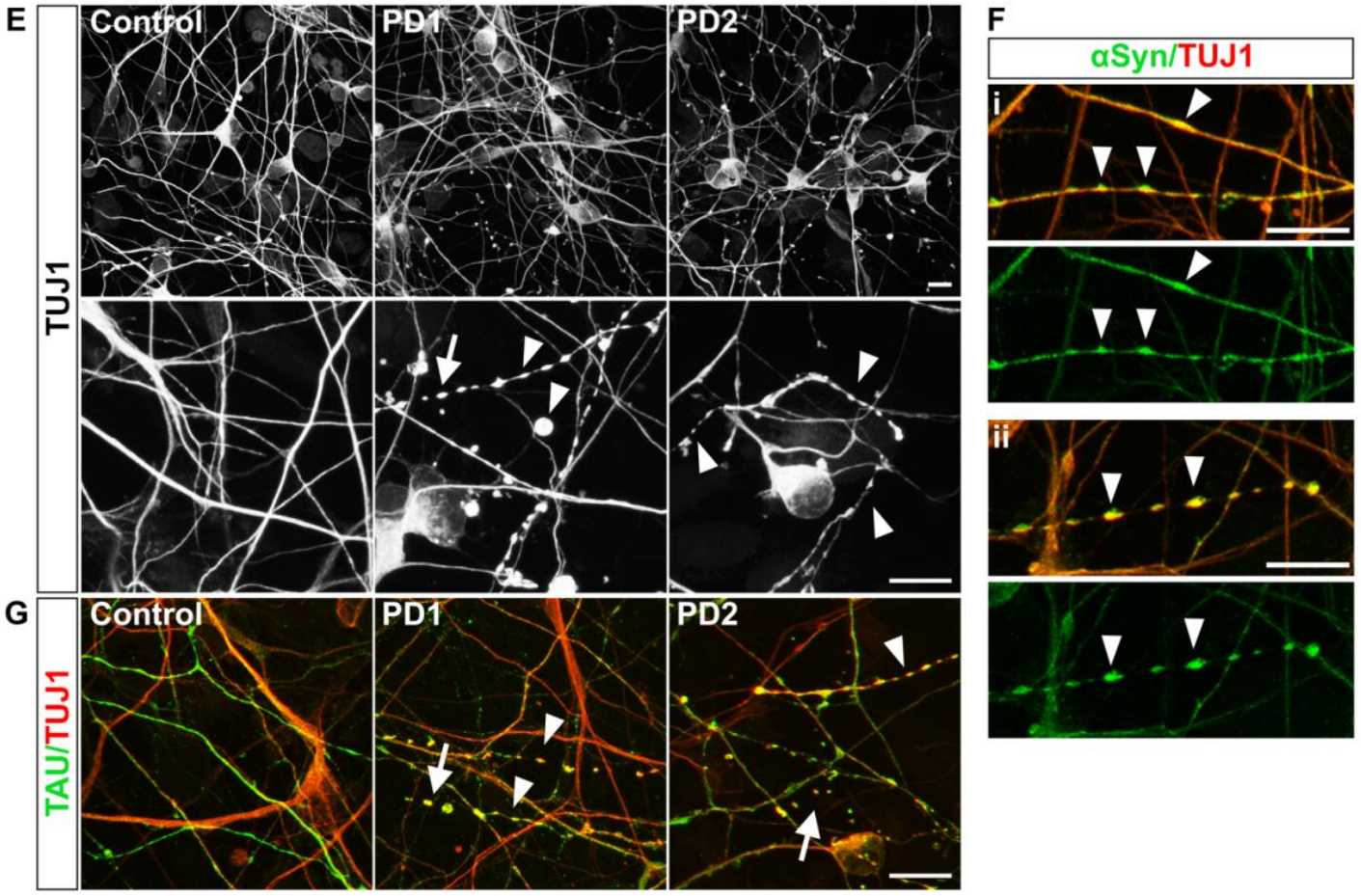
Lewy-like neurites

αSyn aggregates



A53T-neurons displayed disease-relevant phenotypes

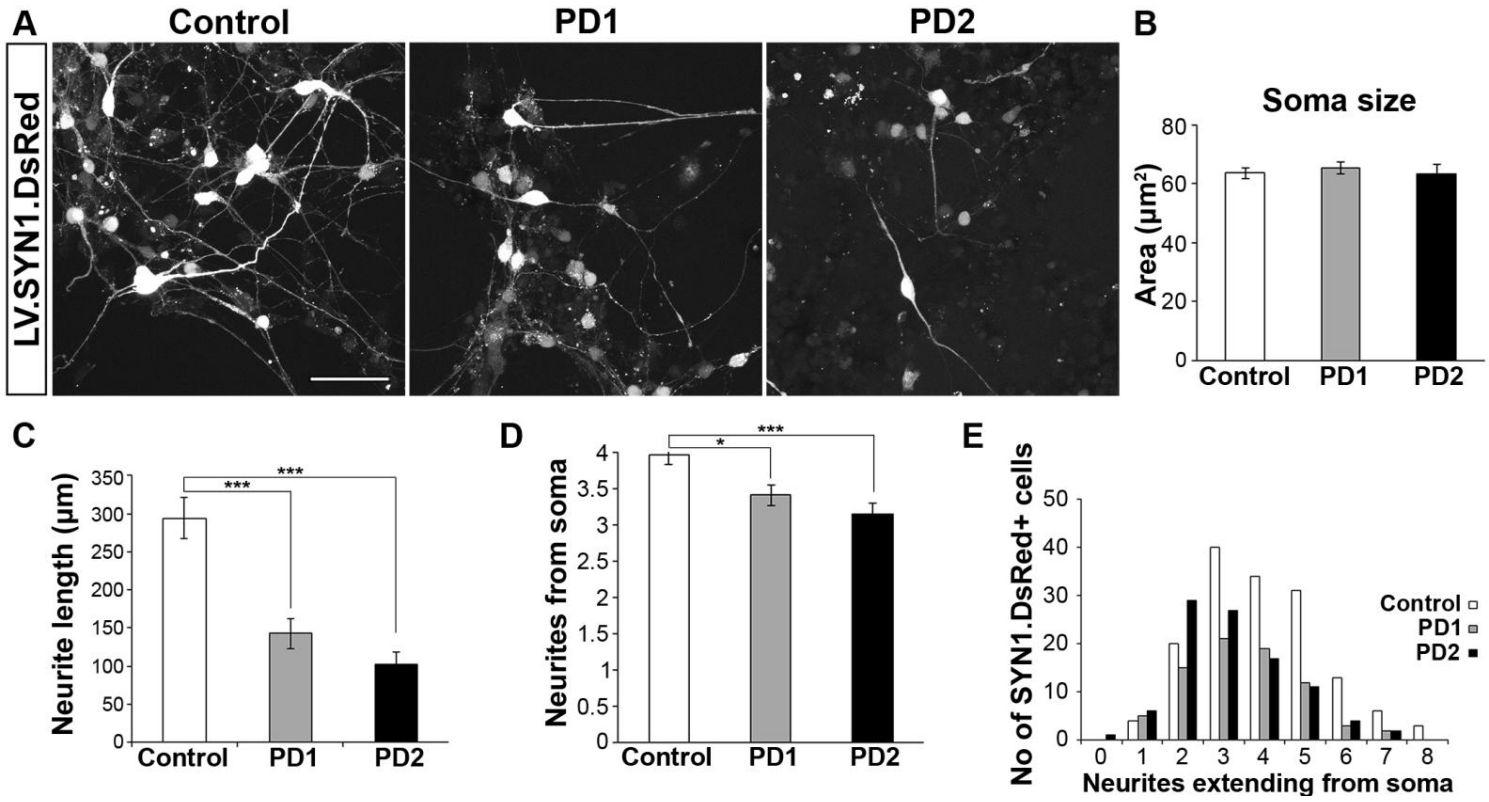
1. protein aggregates, also containing αSyn
2. contorted axons with swollen varicosities containing αSyn and tau



A53T-neurons displayed disease-relevant phenotypes

1. protein aggregates, also containing αSyn
2. contorted axons with swollen varicosities containing αSyn and tau
3. **compromised neuritic outgrowth**

Lentiviral vector for expression of the DsRed under the control of the human synapsin 1 promoter



Transcriptome profiling using RNA-Seq

A53T-dysregulated pathways

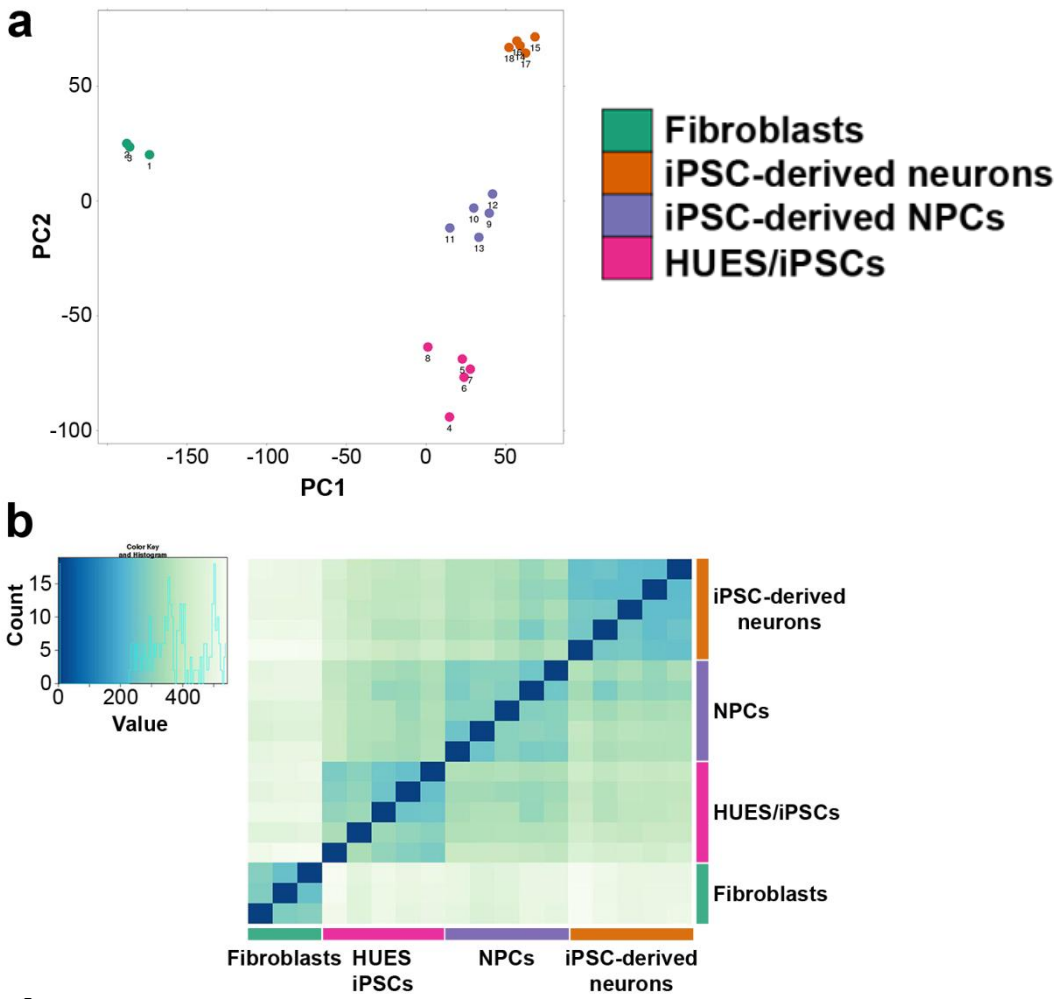
Therapeutic targets

Disease-associated genes

Biomarkers

FIBROBLASTS iPS/ES CELLS NPCs DIFF. NEURONS

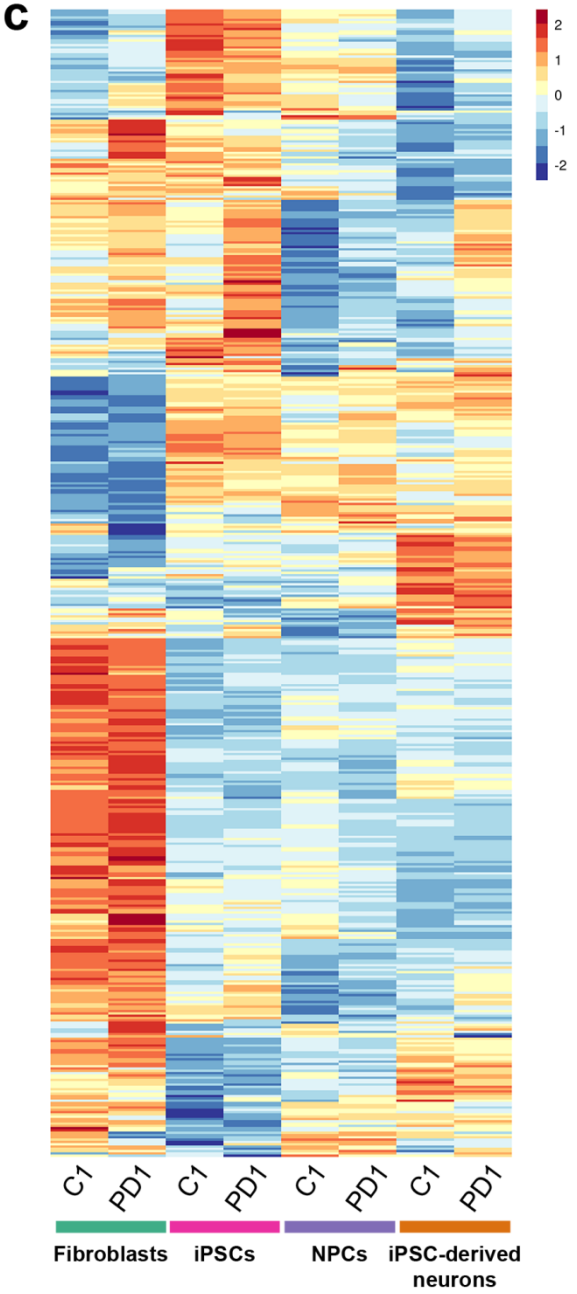
Transcriptome profiling using RNA-Seq



Transcriptome profiling using RNA-Seq

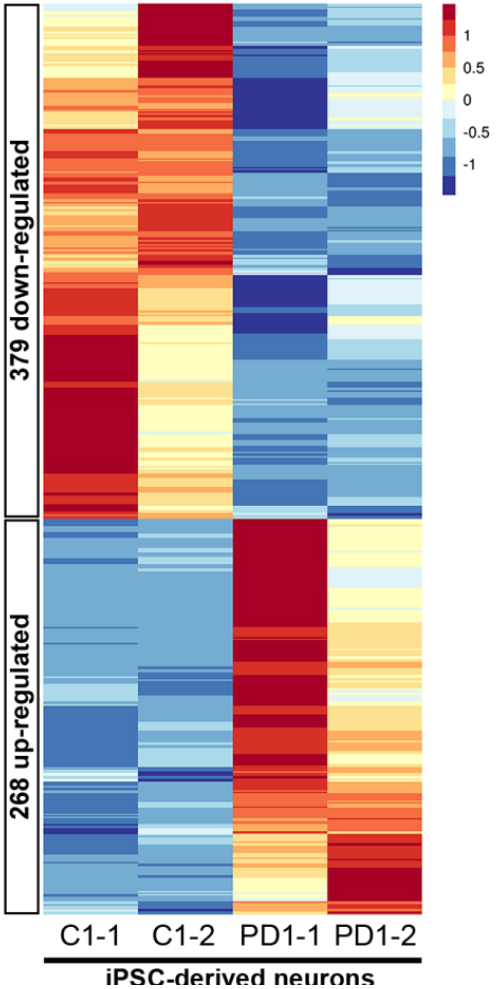
Fibroblasts
iPSC-derived neurons
iPSC-derived NPCs
HUES/iPSCs

Stage of differentiation	Number of mRNA differentialy expressed (PD1 vs Control, p<0.05)
Fibroblast	1094
iPSC	342
NPC	471
Neuron	647



Transcriptome profiling using RNA-Seq

PD vs. CTR iPSC-derived neurons

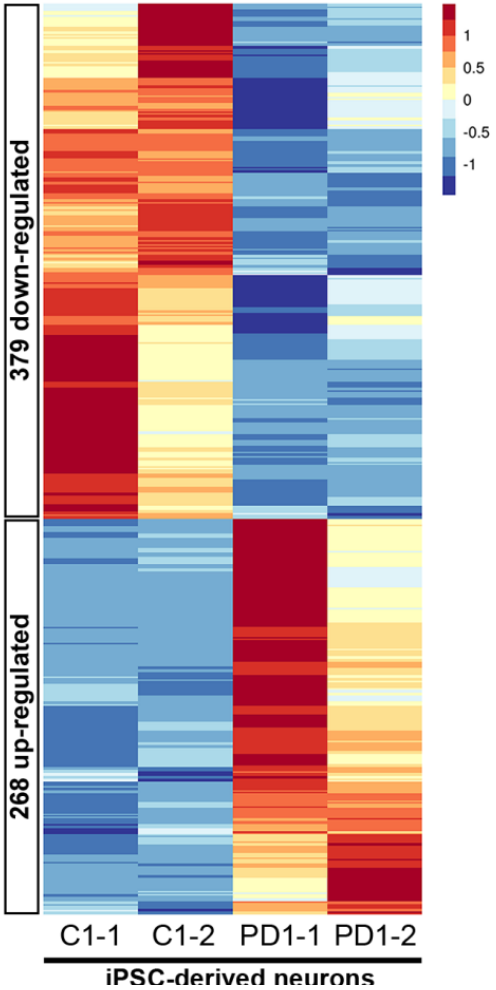


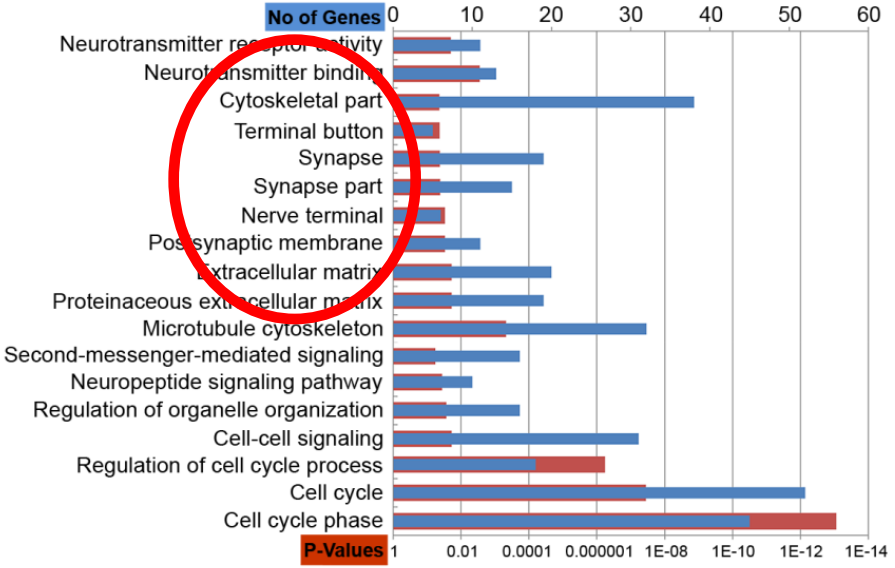
GO analysis

Functional Category	**Number of genes (p<0.05)**
Cell Cycle	48
Transcription/ Translation	47
Metabolism	46
Development/ Differentiation	45
Protein Vesicle/ Trafficking/ Transport	40
Signal Transduction	38
Cell Adhesion and ECM	35
Neuronal	34
Calcium Signaling	27
Immune System	18
DNA Replication/ Repair	15
Unknown	15
Other	15
Protein Modification	13
Cytoskeleton	8
Apoptosis/ Aytophagy	6

Transcriptome profiling using RNA-Seq

PD vs. CTR iPSC-derived neurons





autism

schizophrenia

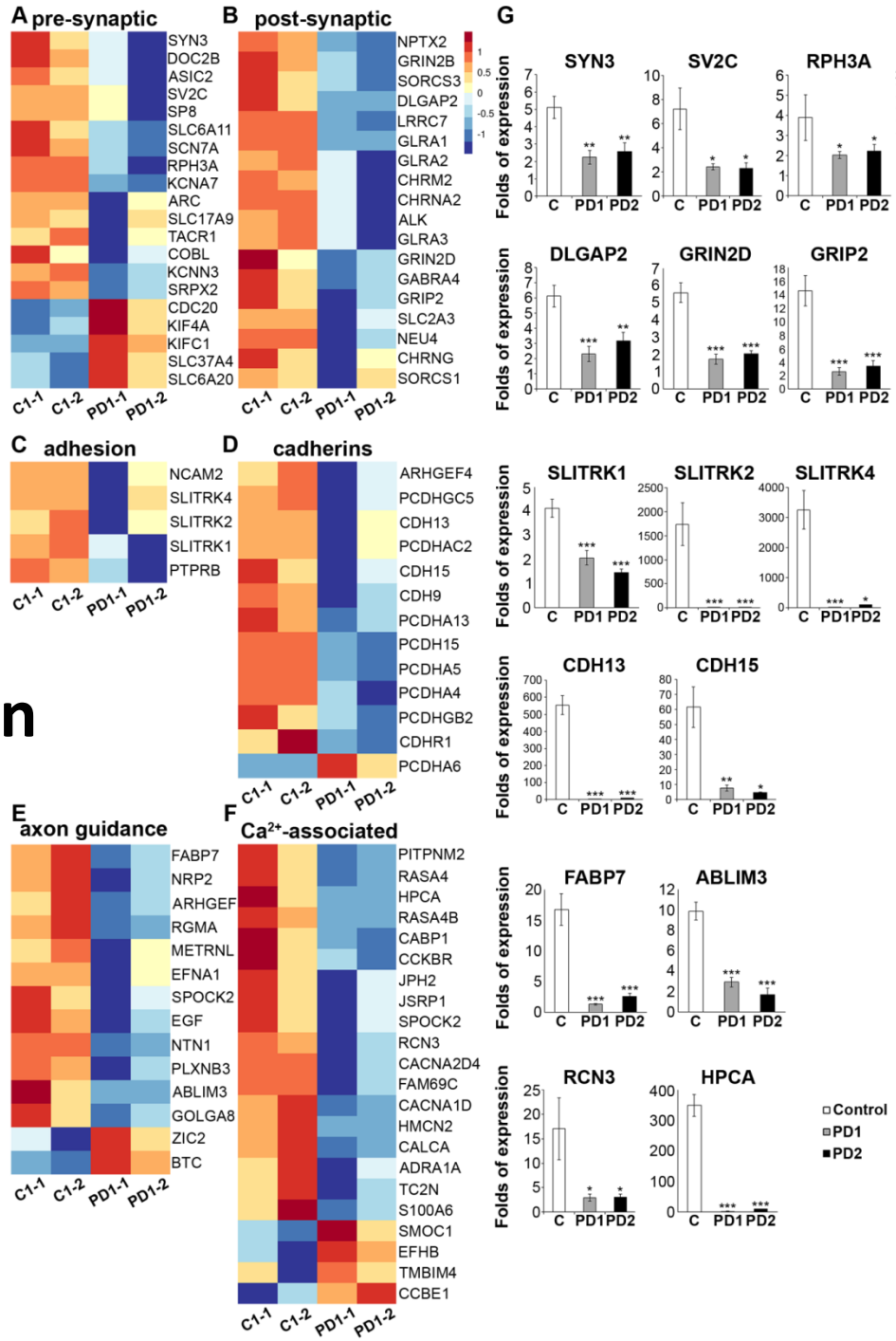
bipolar disorder

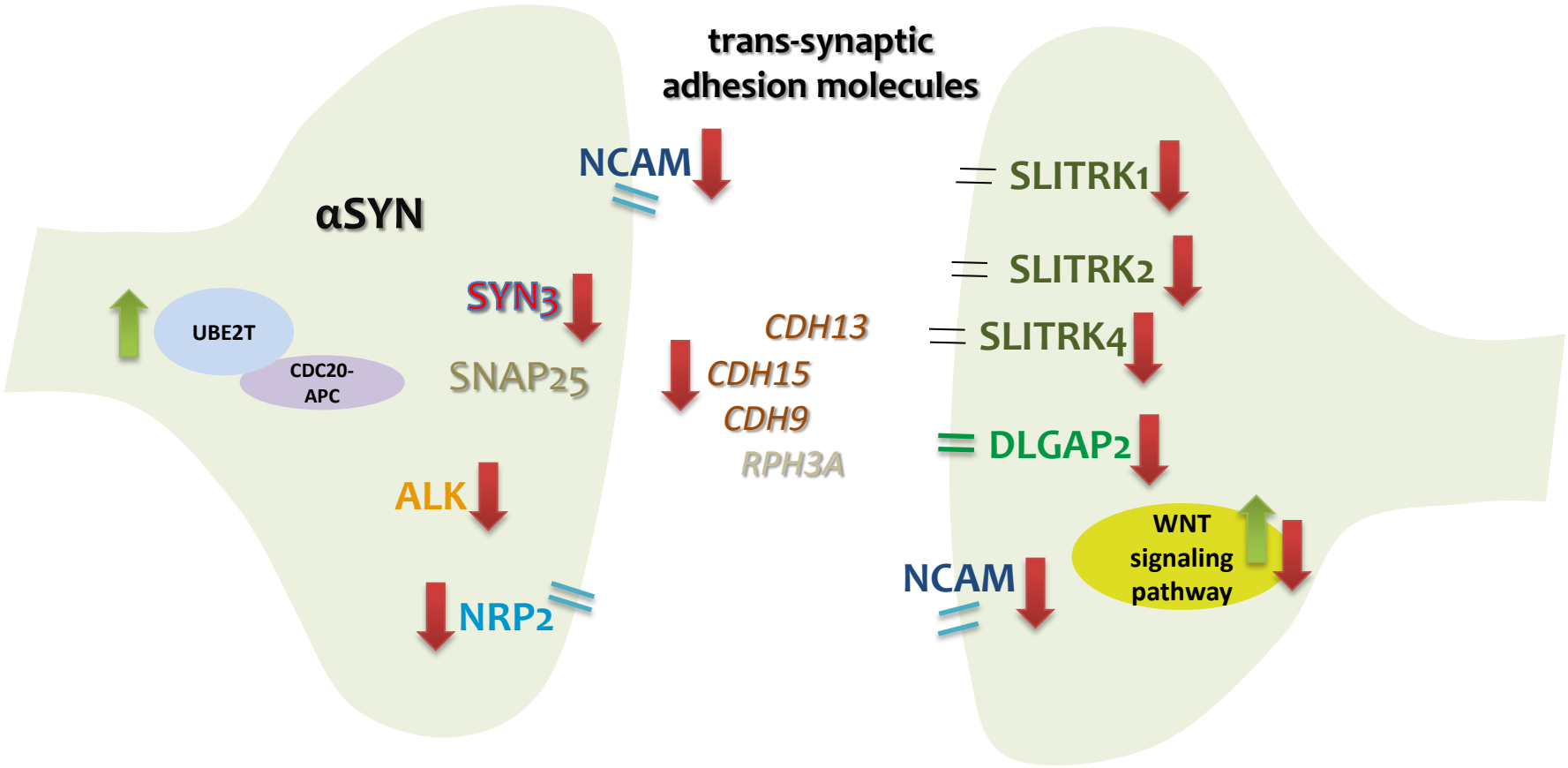
pre-synaptic

post-synaptic

trans-synaptic/ adhesion

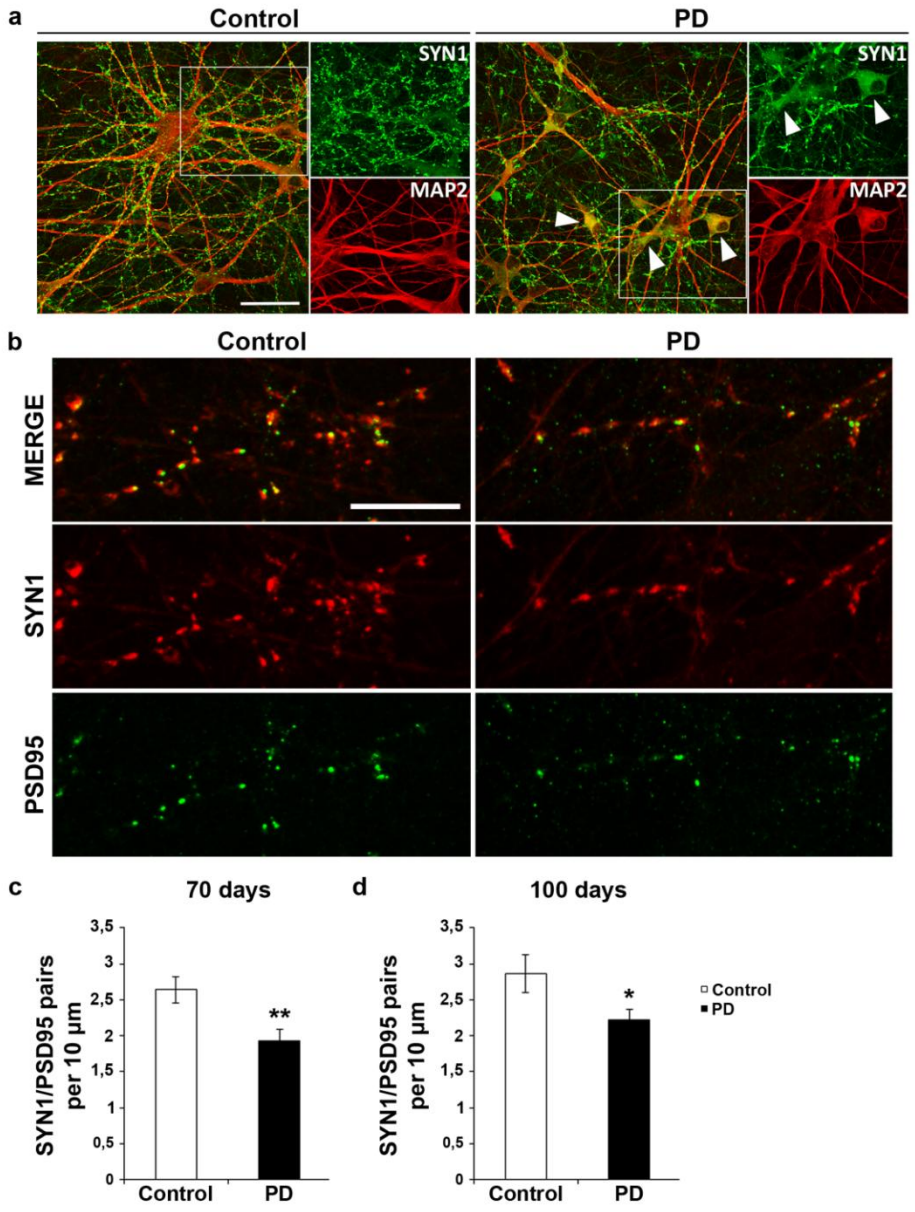
axon guidance



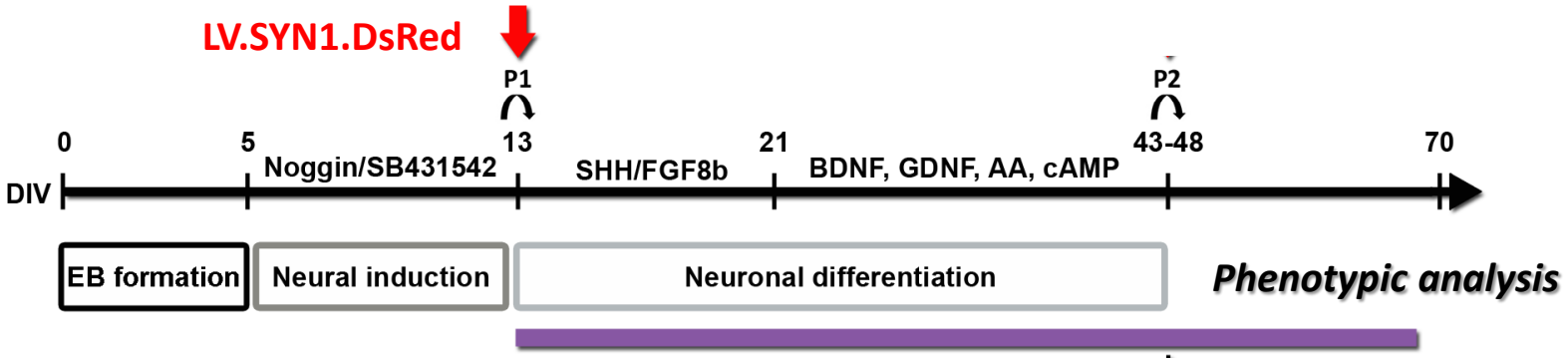

pre-synaptic
post-synaptic
trans-synaptic
adhesion molecules
NCAM
αSYN
SYN3
UBE2T
CDC20-
APC
SNAP25
ALK
NRP2
CDH13
CDH15
CDH9
RPH3A
SLITRK1
SLITRK2
SLITRK4
DLGAP2
WNT
signaling
pathway
NCAM

Reduced synaptic contacts in A53T-neurons

iPSC-derived neurons seeded on mouse astrocytes for up to 100 days

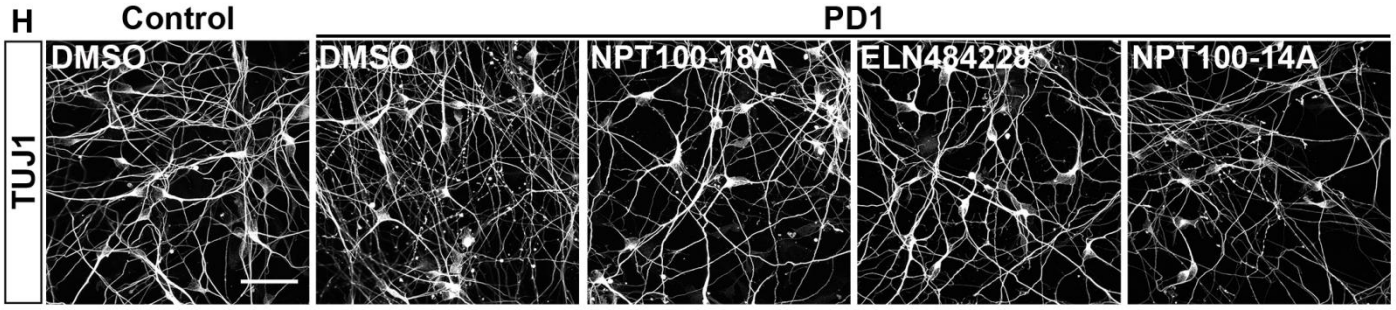


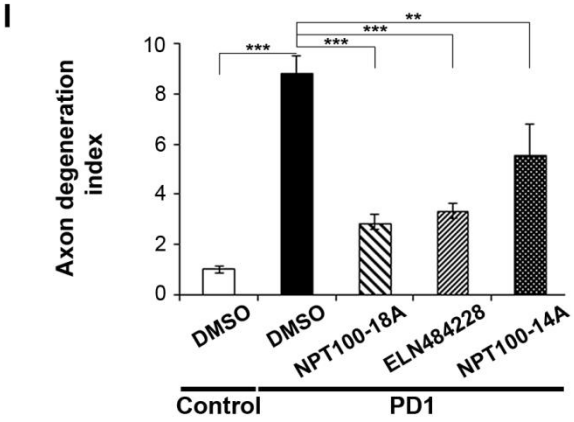
Rescue of pathological phenotype by small molecules targeting αSyn



Small molecule inhibitors of αSyn aggregation (Dr E. Masliah, UCSD)

Wrasidlo W, et al., Brain 2016

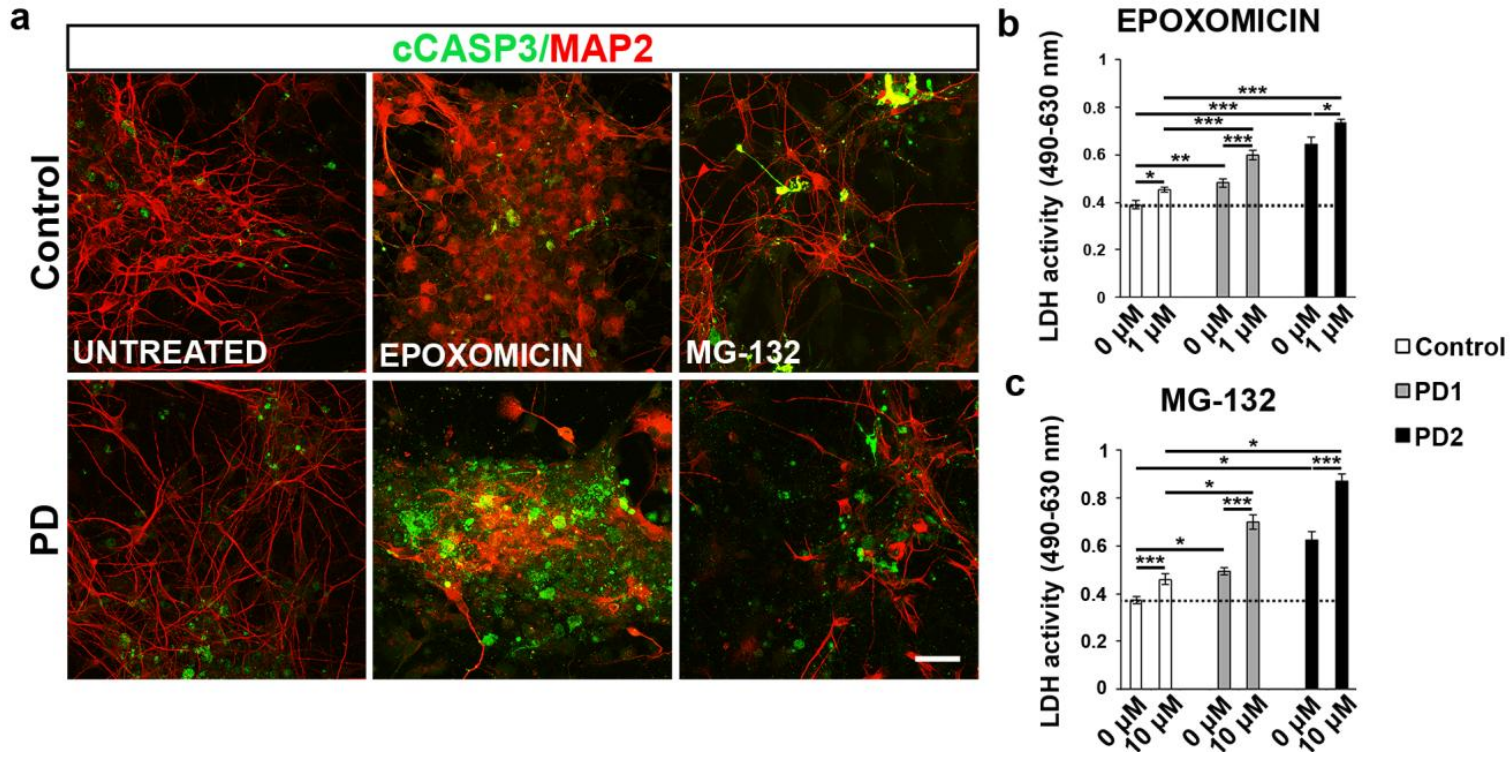




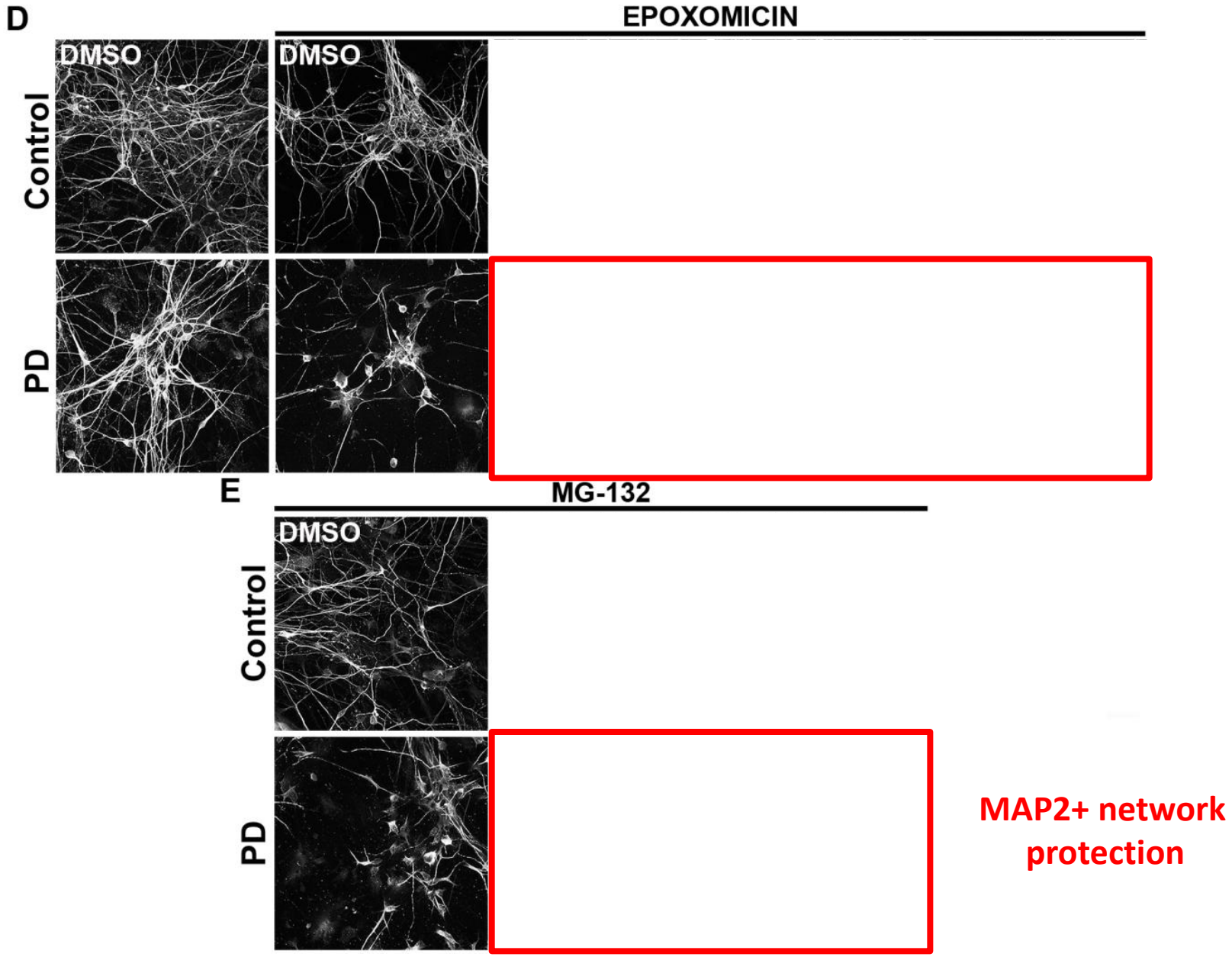
3. Alleviate significantly the existence of distorted/degenerating axons

Are the small molecules effective under induced stress conditions?

PD neurons: increased susceptibility to environmental stress conditions



Reversal of induced-stress phenotypes by small molecules targeting αSyn



MAP2+ network protection

Conclusions

An iPSC-based model that…

1. faithfully simulates disease pathogenesis and uncovers novel disease-relevant phenotypes at basal conditions

protein aggregation

compromised neuritic outgrowth

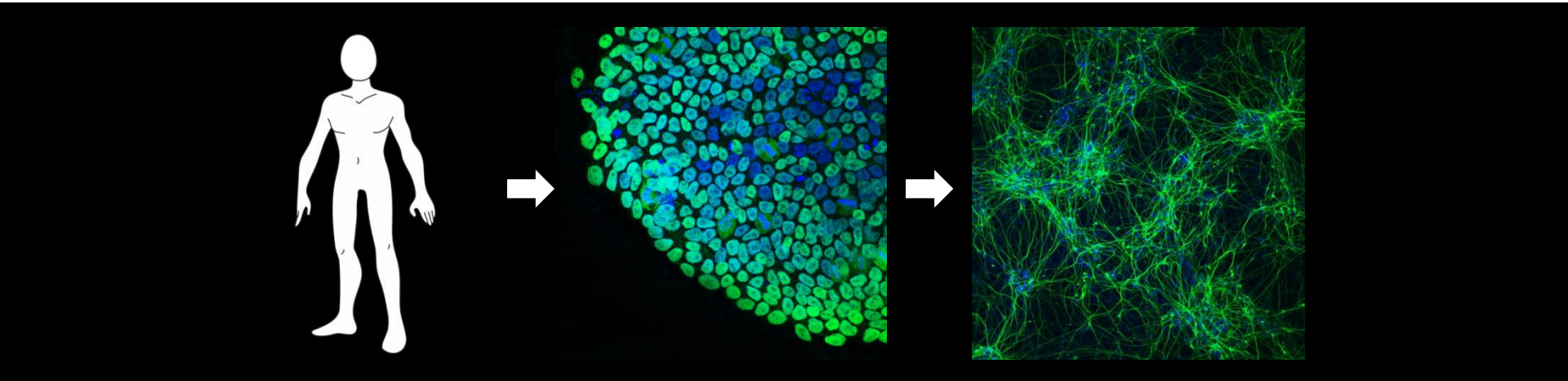
axonal αSyn/tau-associated pathology

alterations in synaptic molecules

2. highlights a feasible therapeutic approach (protective effects of small-molecule inhibitors of αSyn aggregation)

3. can be used as a platform to screen disease-modifying drugs

Kouroupi et al, PNAS 2017 in press



Cellular and Molecular Neurobiology Lab, IP-Athens

Rebecca Matsas
Era Taoufik
Florentia Papastefanaki
Nasia Antoniou
Rania Zygogianni
Maria Gaitanou
Nelly Prodromidou
Katerina Segklia
Nikos Kokkorakis
Konstantinos Tsioras

Collaborators

Artemis Hatzigeorgiou, DIANA-Lab
Yannis Vlachos, DIANA-Lab
Piotr Bregestovski, Aix-Marseille University
Dafni Chroni-Tzartou, IP-Athens
Delphine Bohl, IP-Paris
Eliezer Masliah, UCSD
Wolf Wrasidlo, UCSD

Leonidas Stefanis, BRFAA
Kostas Vekrellis, BRFAA
Panagiotis Politis, BRFAA
Dimitris Stellas, BRFAA

PTR 417 & 523
Institut Pasteur
FONDATION SANTÉ
ARISTEIA
ParkinsonTransMed
NoPLus
NeuroSign