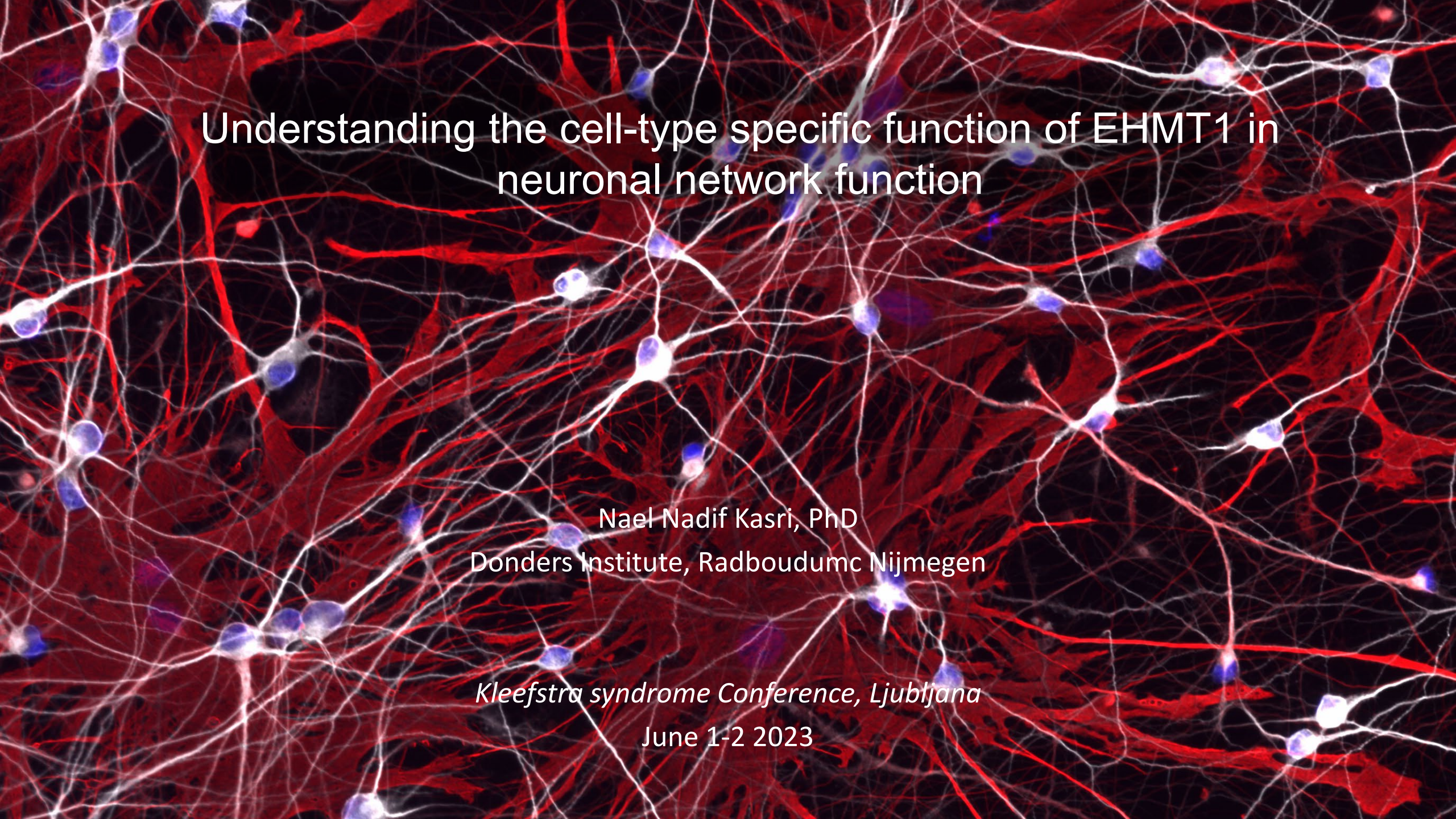
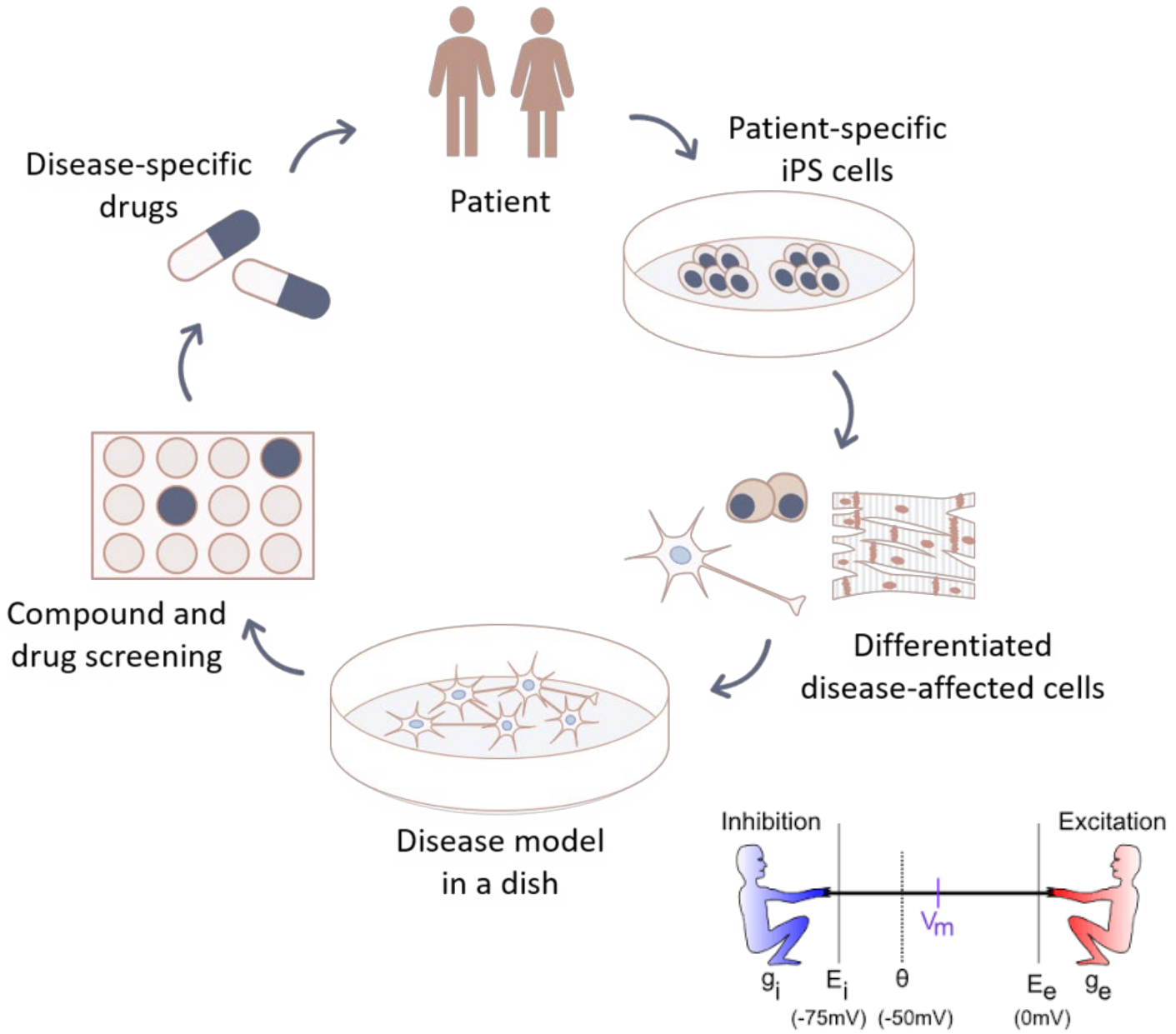


Understanding the cell-type specific function of EHMT1 in neuronal network function

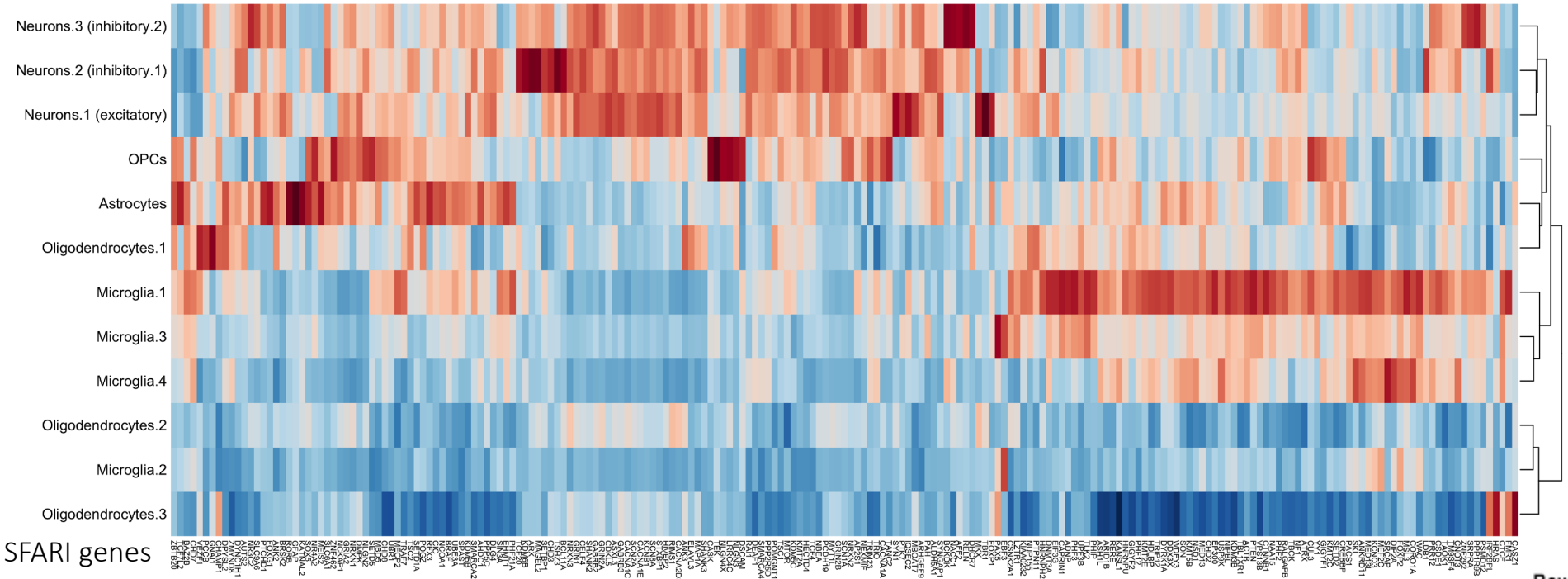
Nael Nadif Kasri, PhD
Donders Institute, Radboudumc Nijmegen

Kleefstra syndrome Conference, Ljubljana
June 1-2 2023

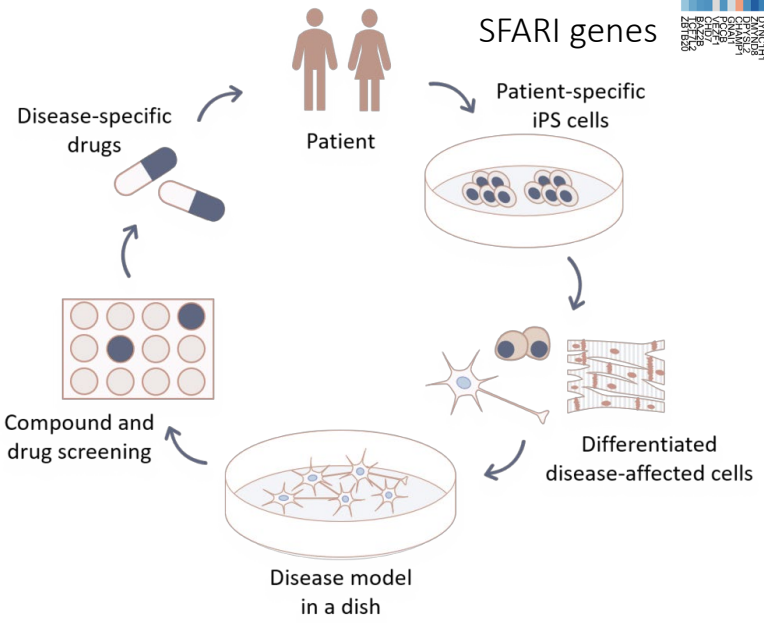
iPSC-derived models for translational studies: **Opportunities and challenges**



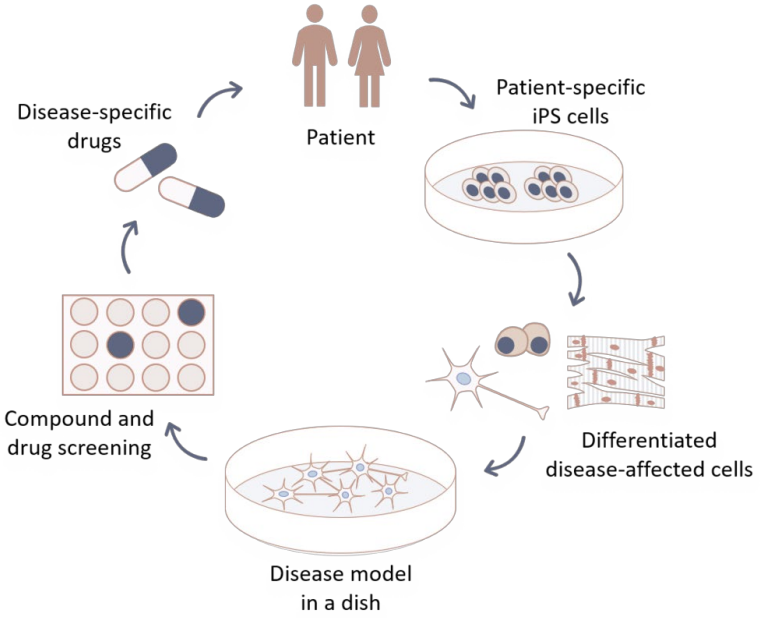
iPSC-derived models for translational studies: **Opportunities and challenges**

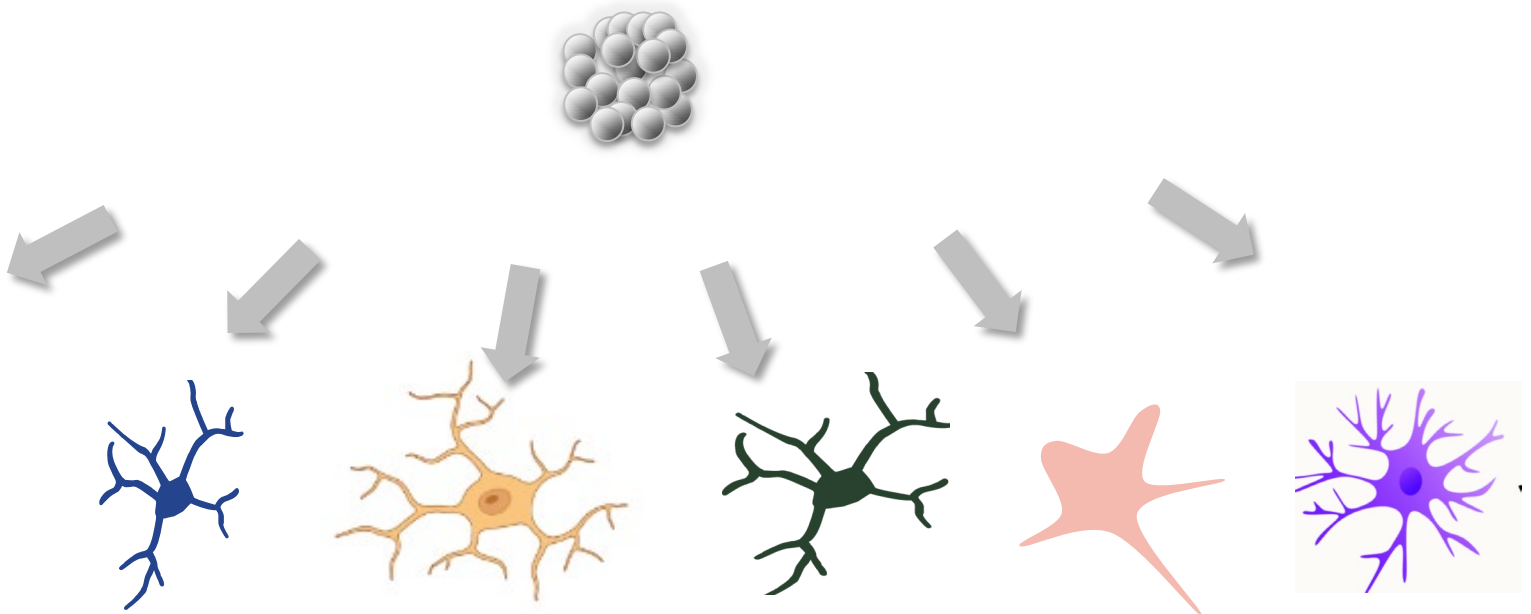


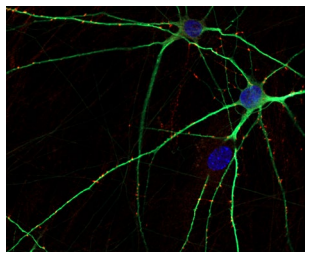
L. de Wit, *unpublished*



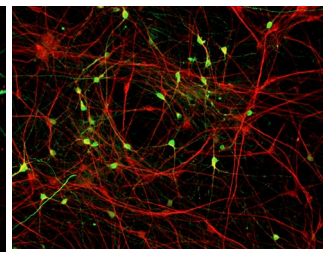
iPSC-derived models for translational studies: **directed differentiation**



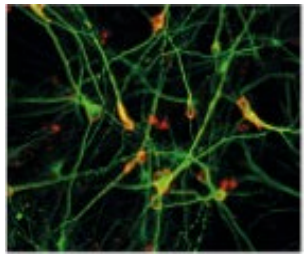




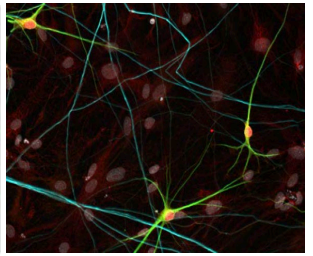
Frega et al, Nat comm, 2019



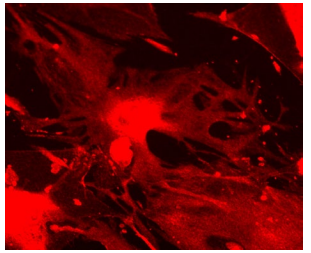
Mossink et al., Mol psych, 2022



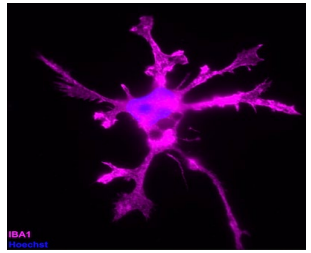
Van Rhijn et al., Neurobiol Dis, 2022



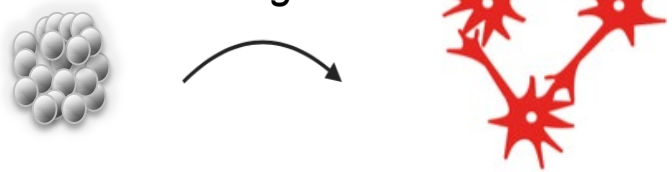
Hesen et al., in prep



Lendemeijer et al, BioRxiv



Mordelt et al, in prep

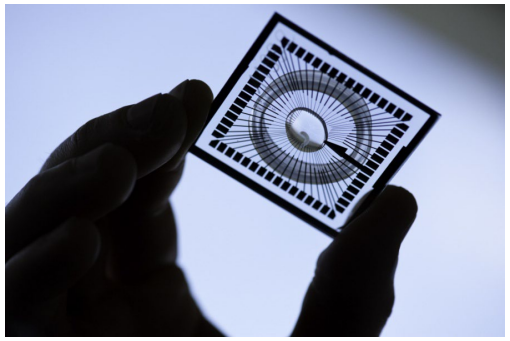


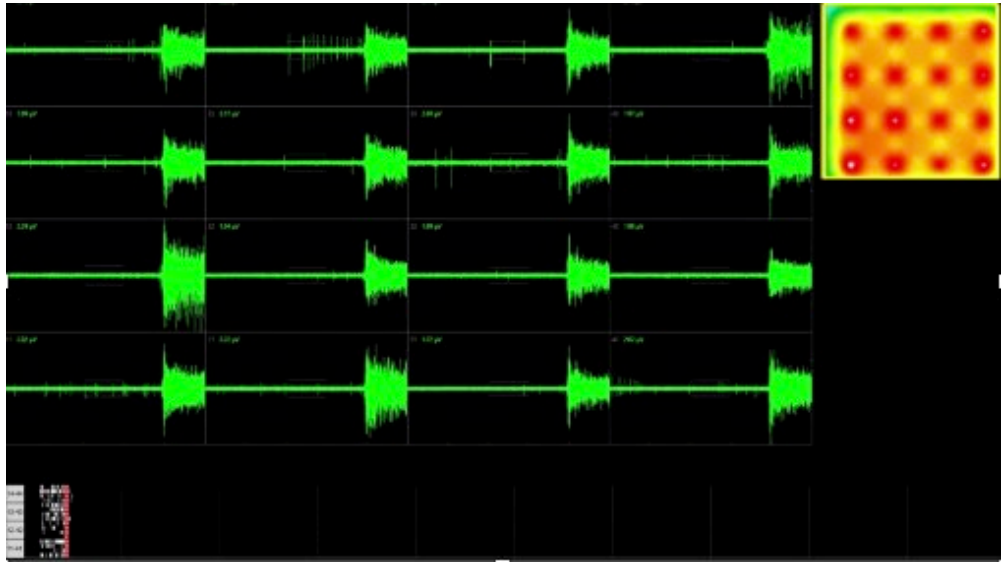
Frega et al, JOVE, 2017

- Fast protocol ± 3 weeks
- High efficiency
- Mature neurons
- Homogeneous population
- Glia dependent

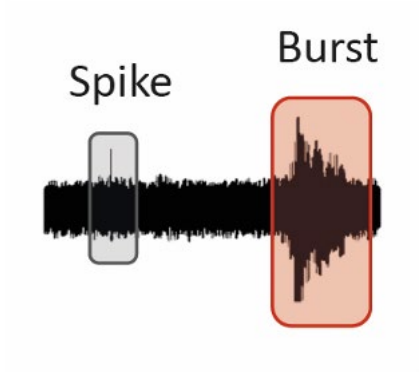
Measuring neural network activity of iPSC-derived networks

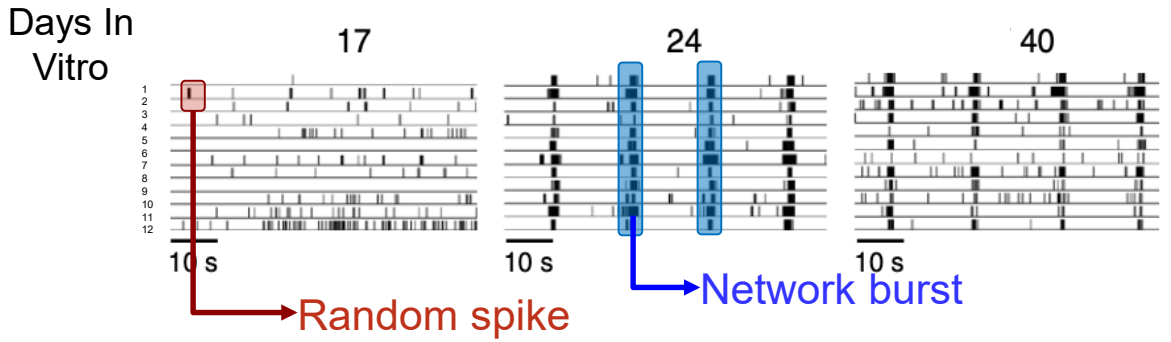
Micro-electrode arrays





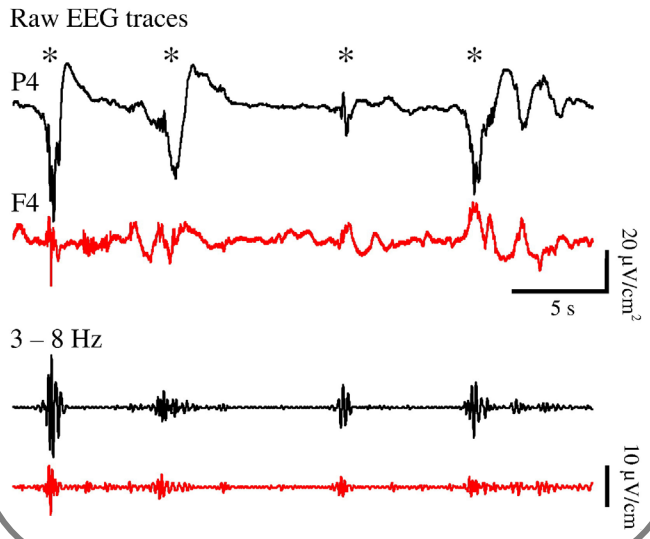
Development



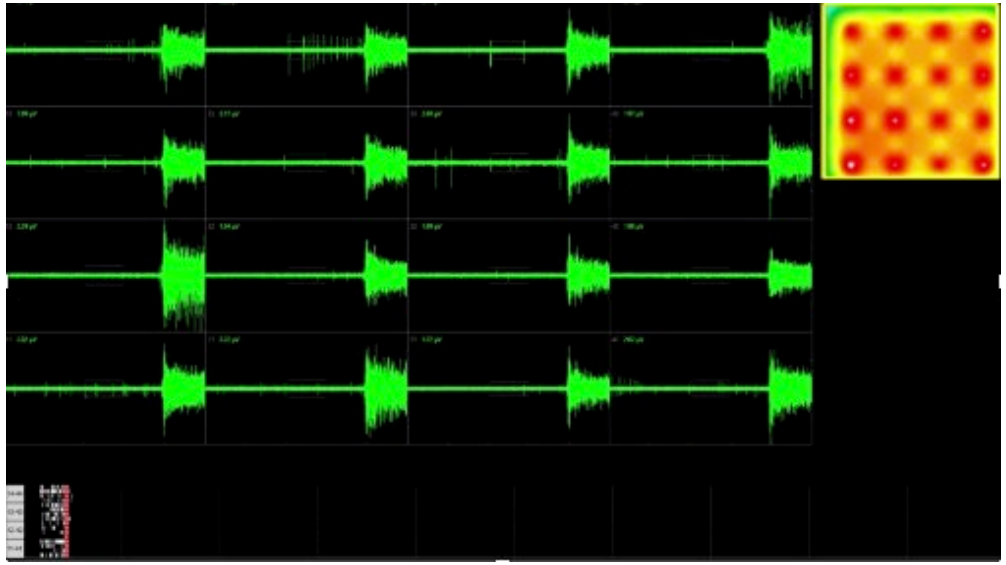


Mossink et al., *Stem Cell Rep 2021*

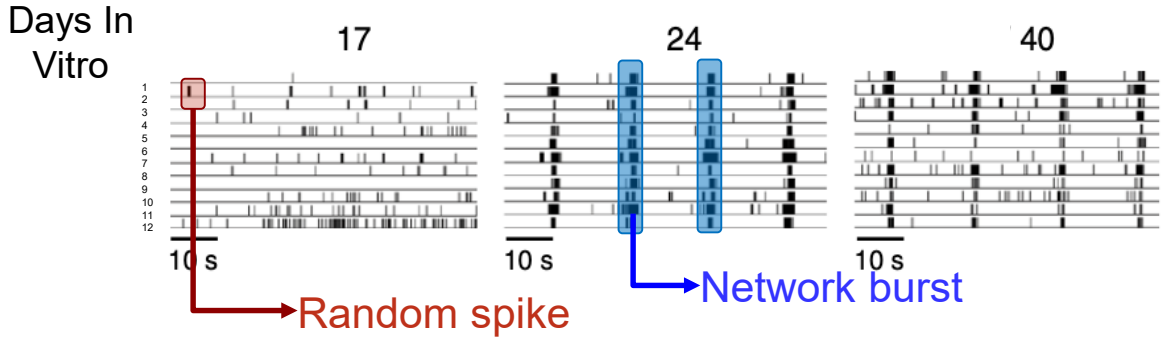
Measuring neural network activity of iPSC-derived networks



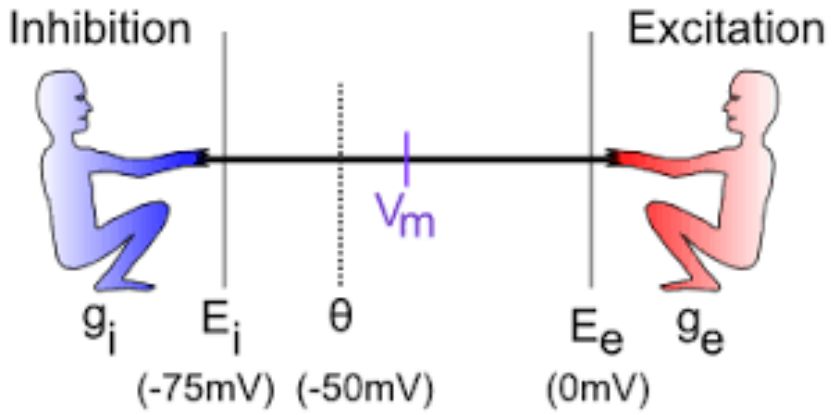
Synchronized Burst are observed in human brain

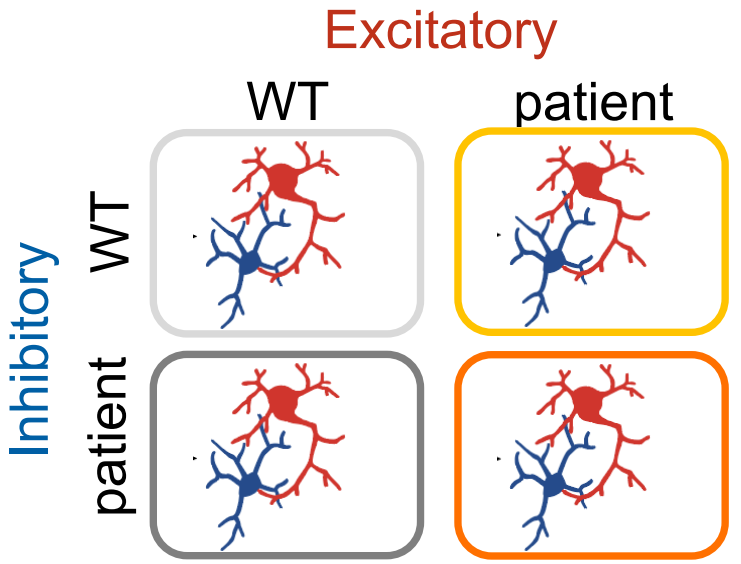


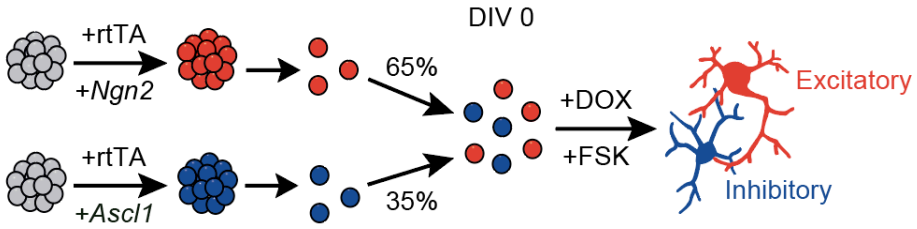
Development

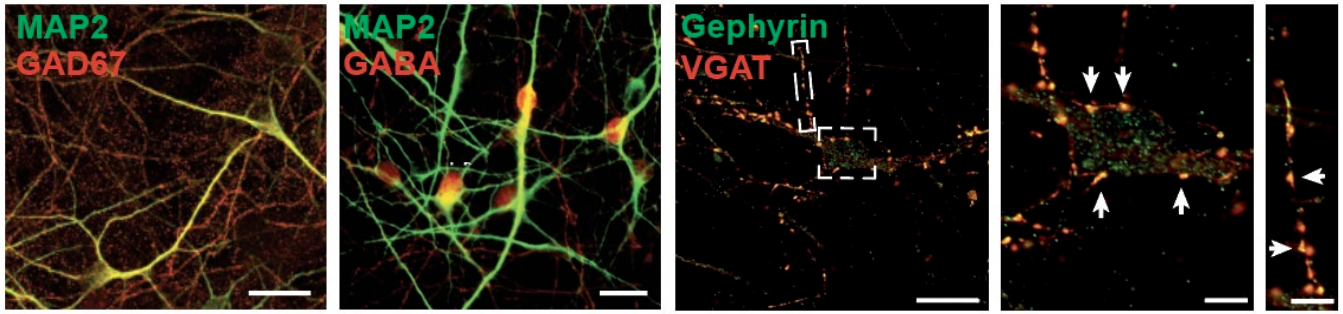


Probing for E/I balance in human pluripotent stem cell-derived neuronal networks



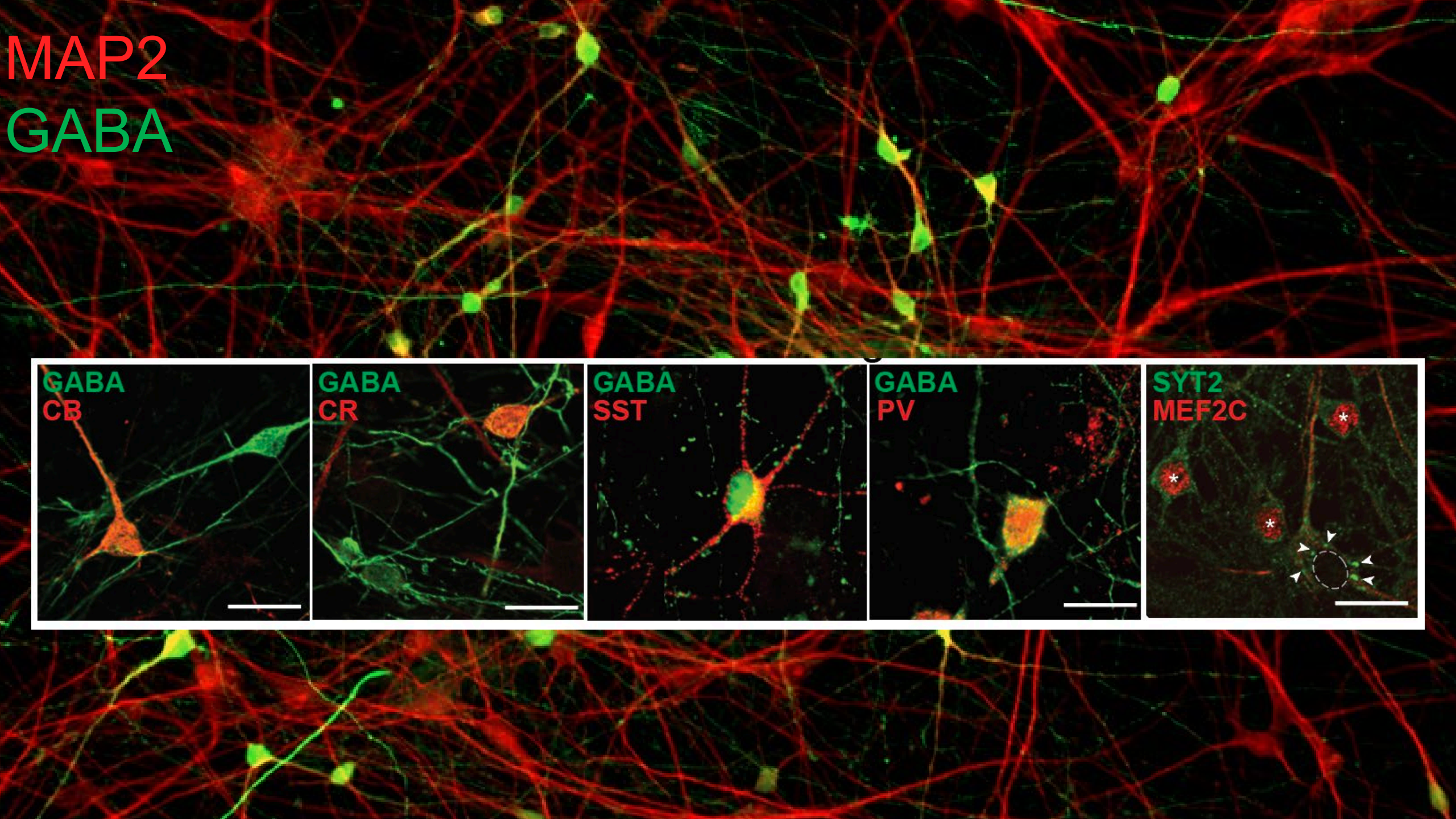




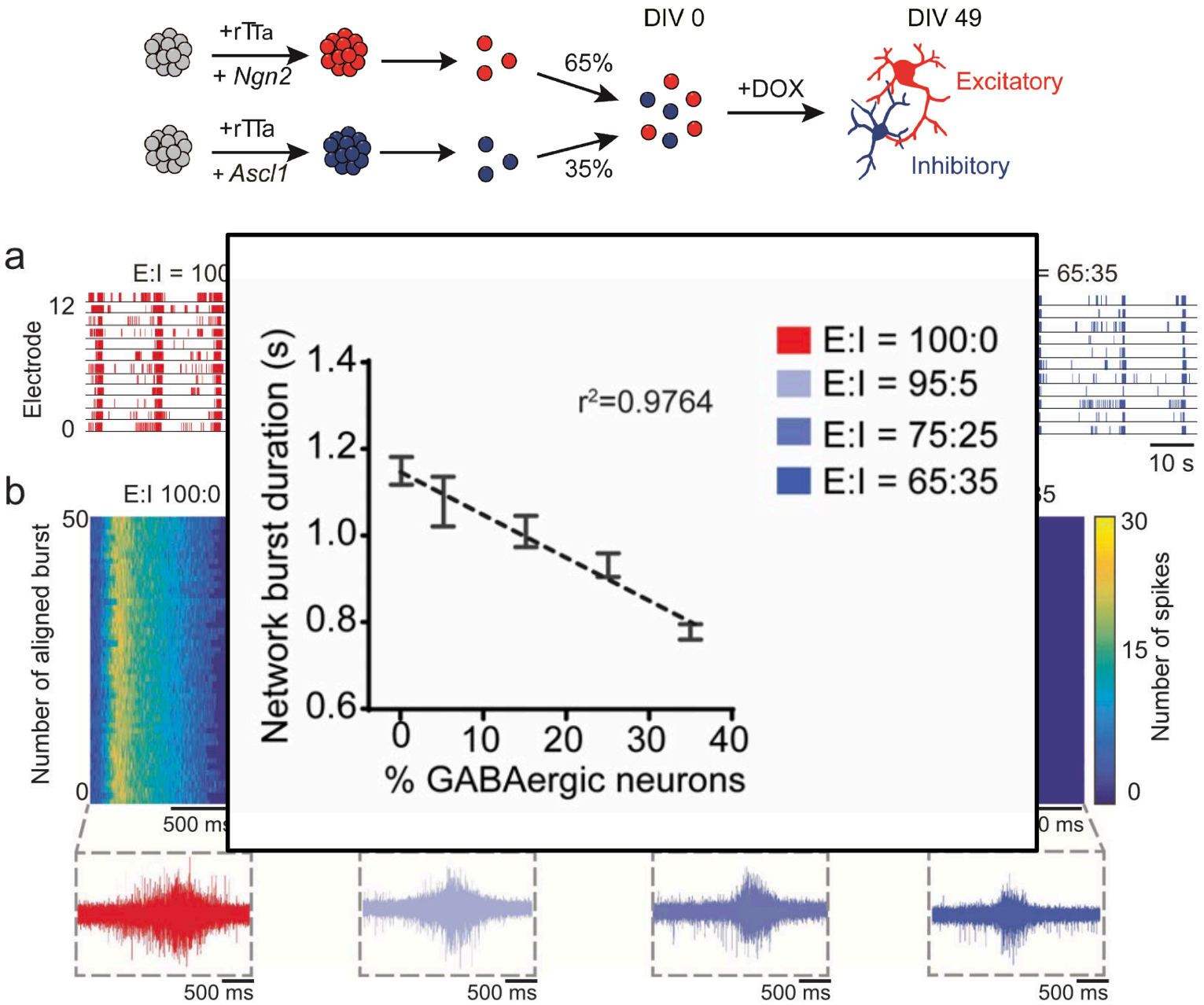


- Adapted from Yang et al., 2017
- Adapted from Shi, Z., et al., 2016

Mossink et. al, Mol Psych , 2022


MAP2
GABA
GABA
CB
GABA
CR
GABA
SST
GABA
PV
SYT2
MEF2C

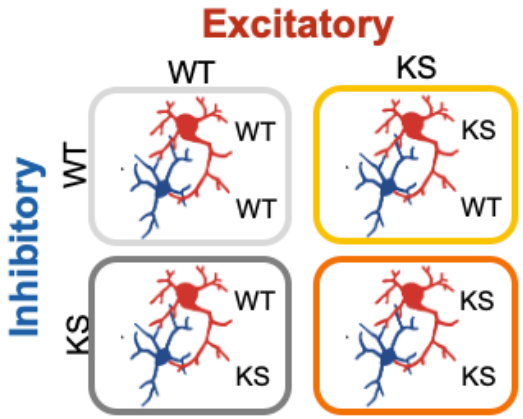
iGABAergic neurons exhibit scalable functional inhibitory control

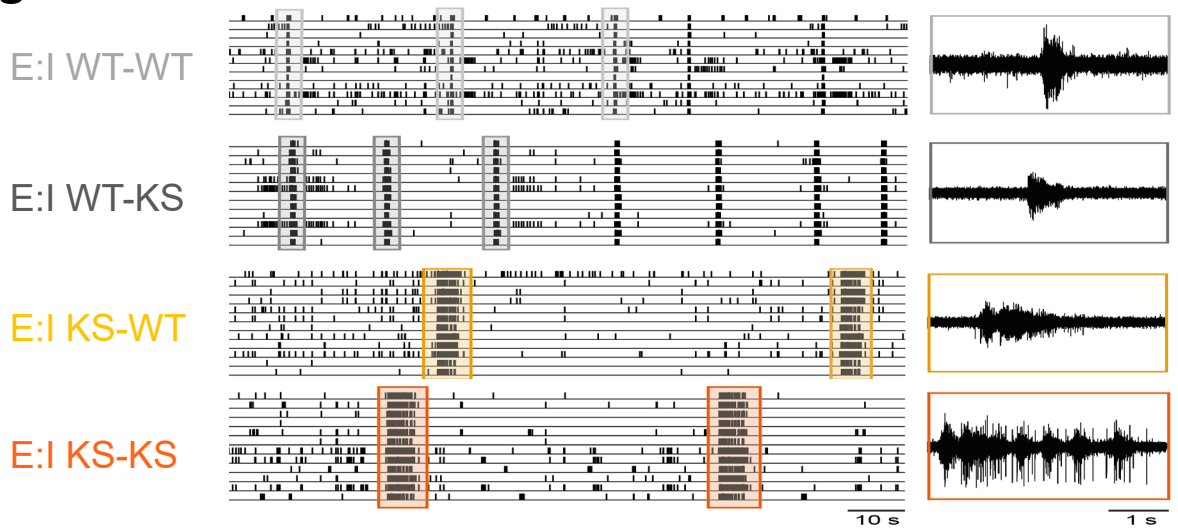


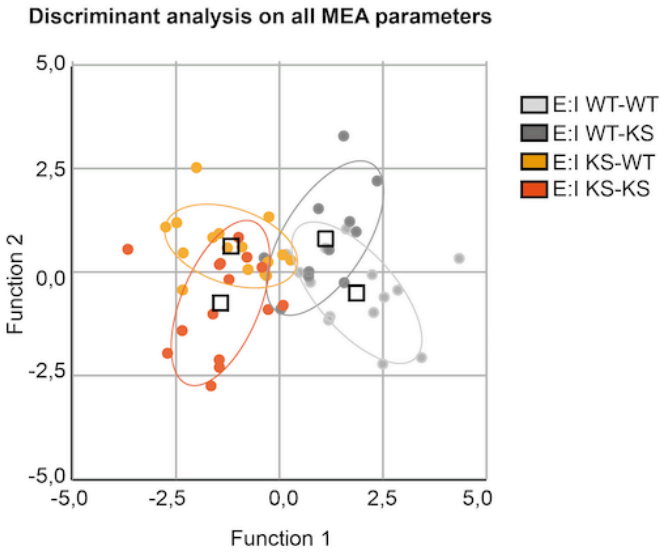
Mossink et. al, Mol Psych , 2022

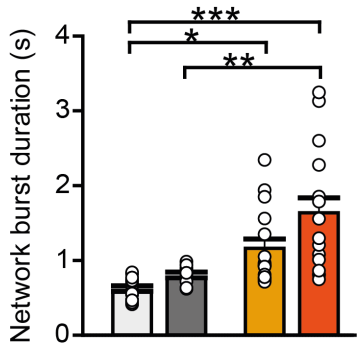
Contribution of excitation and inhibition to **Kleefstra syndrome** neuronal network phenotypes

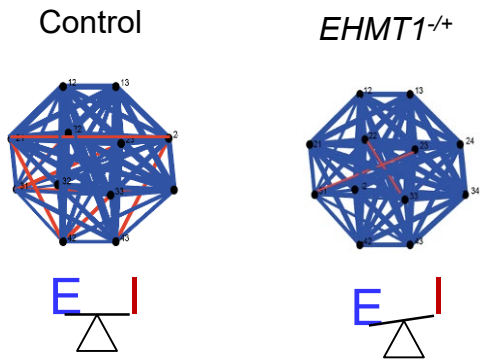
KS: Haploinsufficiency of the *EHMT1* gene







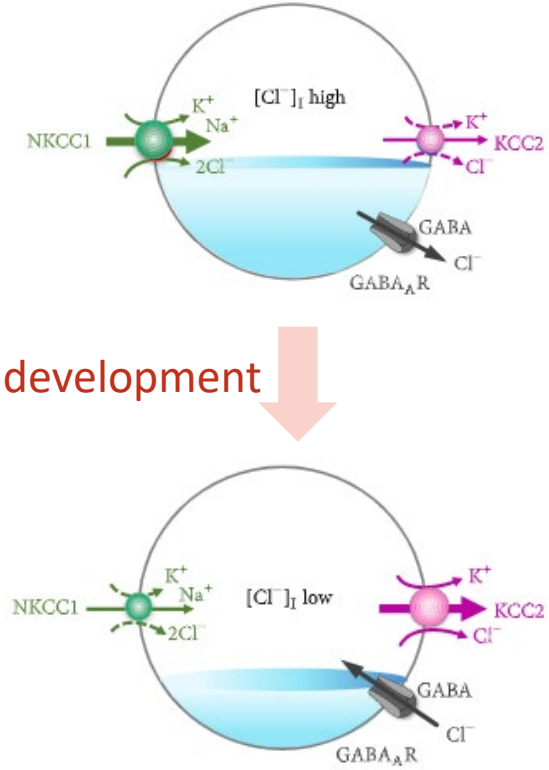




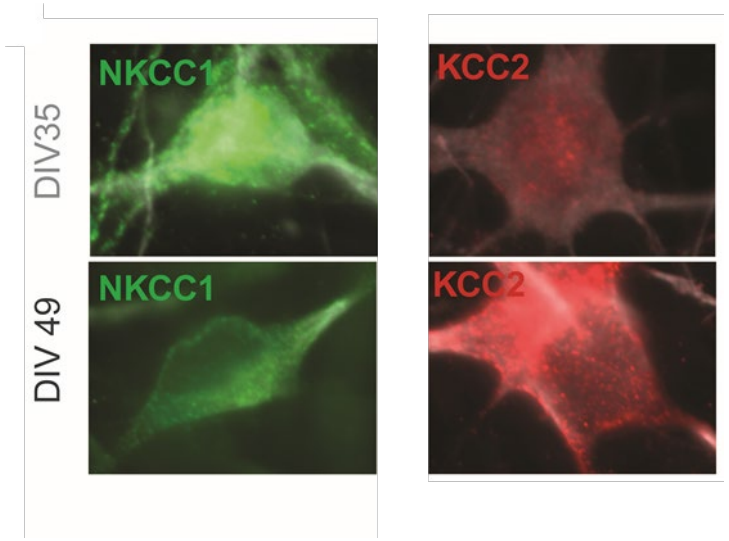
Excitatory links Inhibitory links

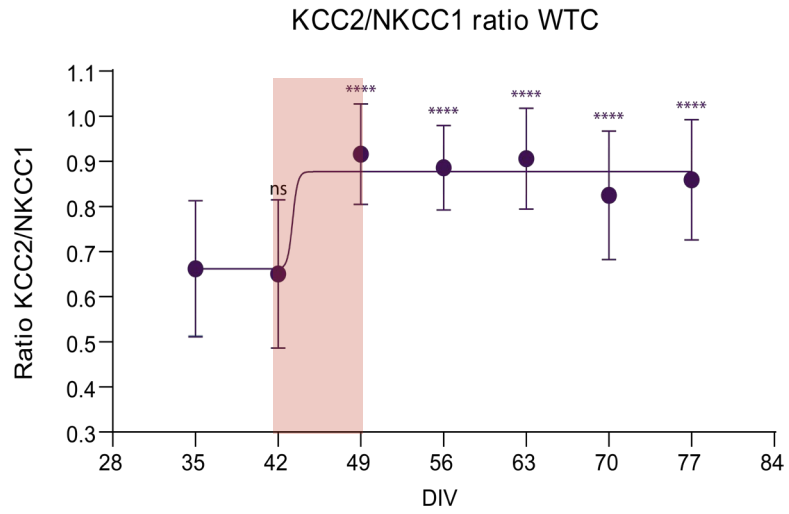
Reduced Inhibition

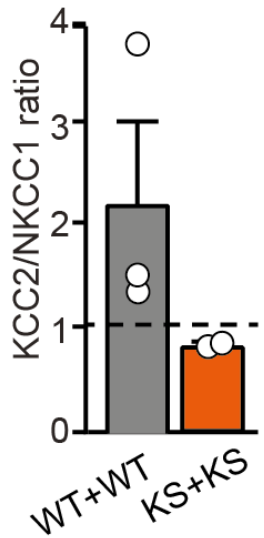
Delayed GABAergic maturation in Kleefstra neuronal networks

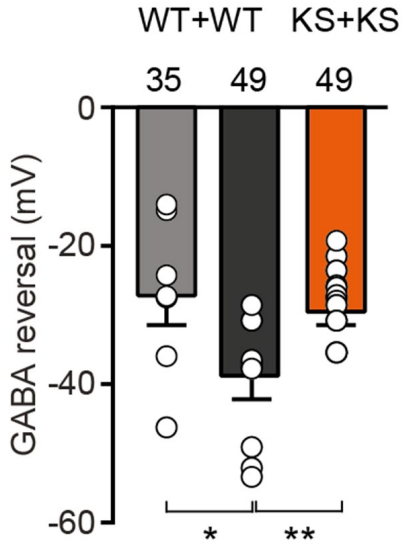


KCC2/NKCC1 expression

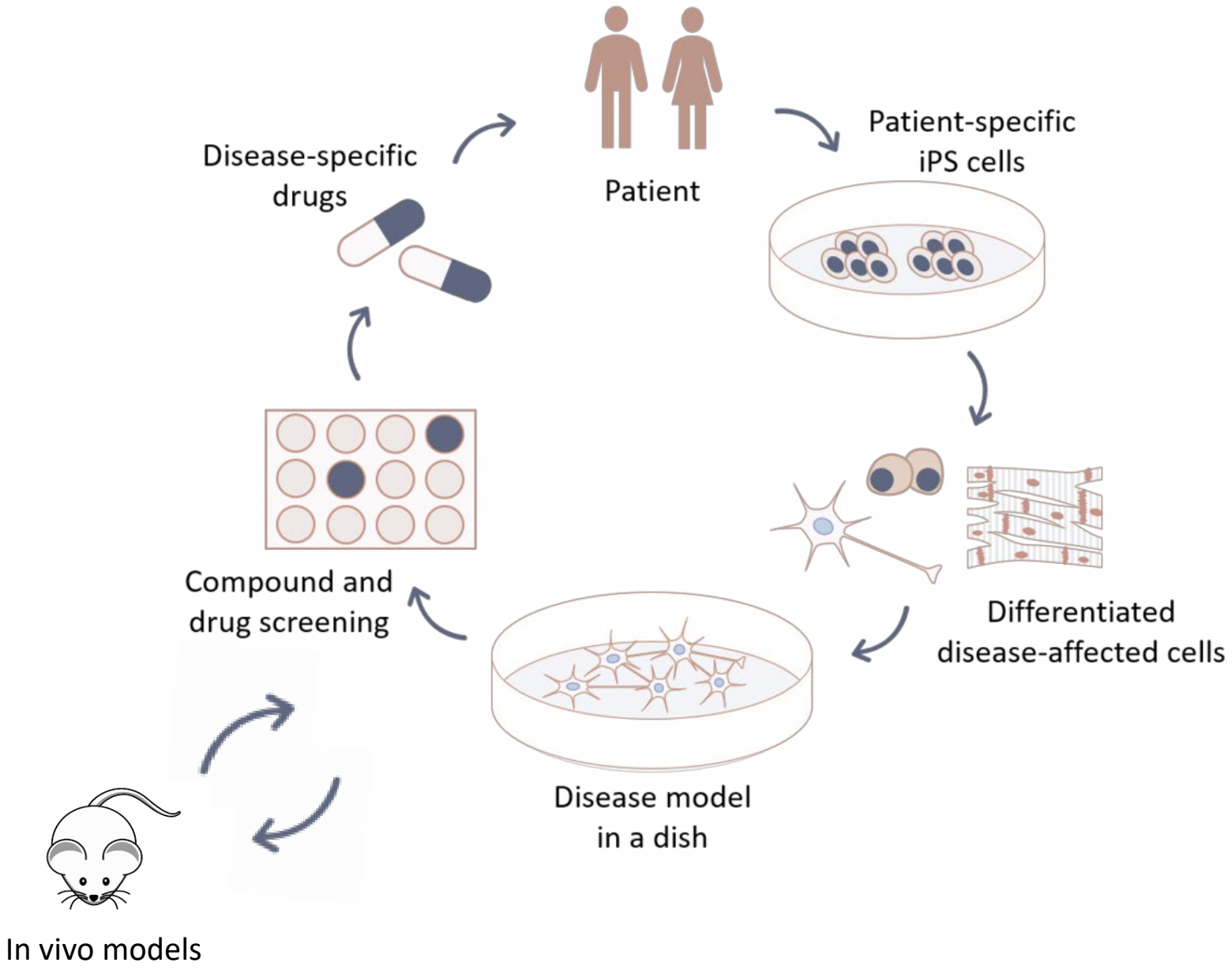




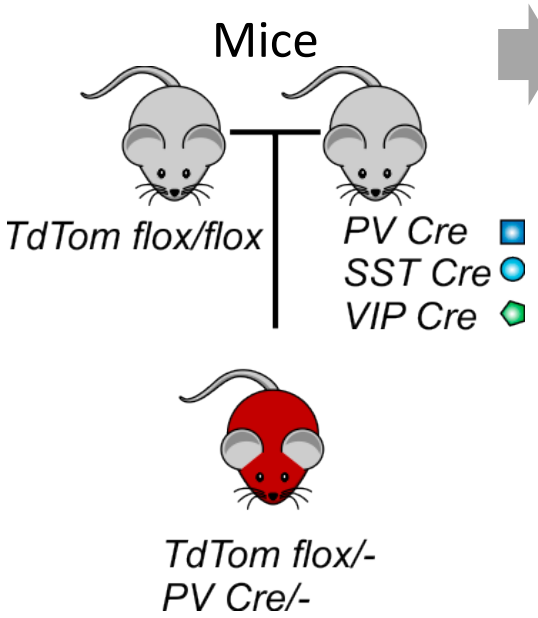


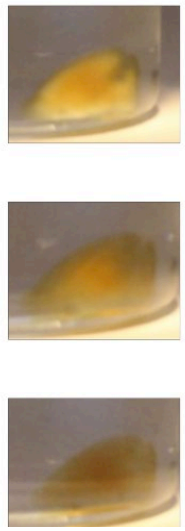


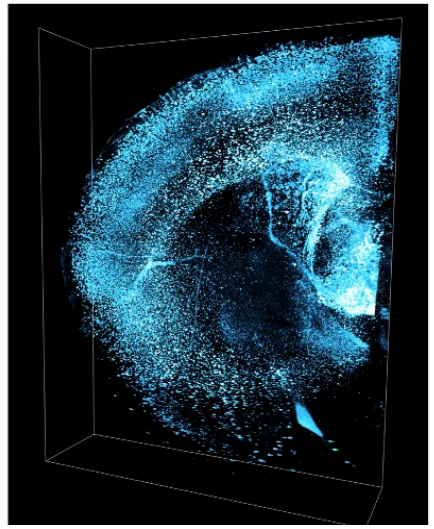
How do findings in a dish translate to *in vivo*?



Altered development of GABAergic neurons in *EHMT1*$^{+/-}$ mice?



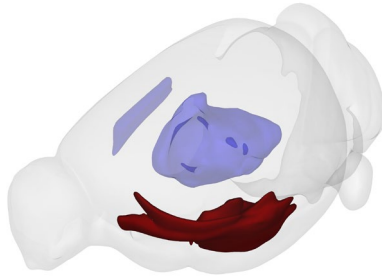




Analysis: FriendlyClearMap

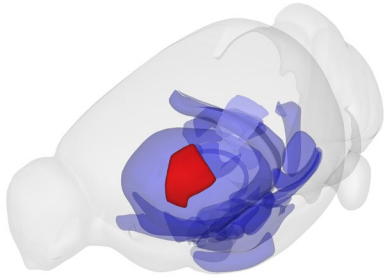
Negwer M et al., 2023 *GigaScience*

PV



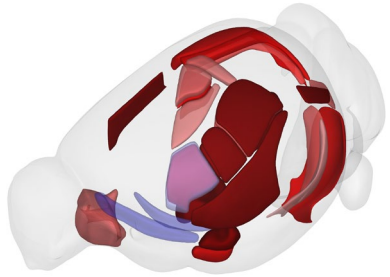
Increased PV in Amygdala

SST



Decreased VIP in Amygdala

VIP



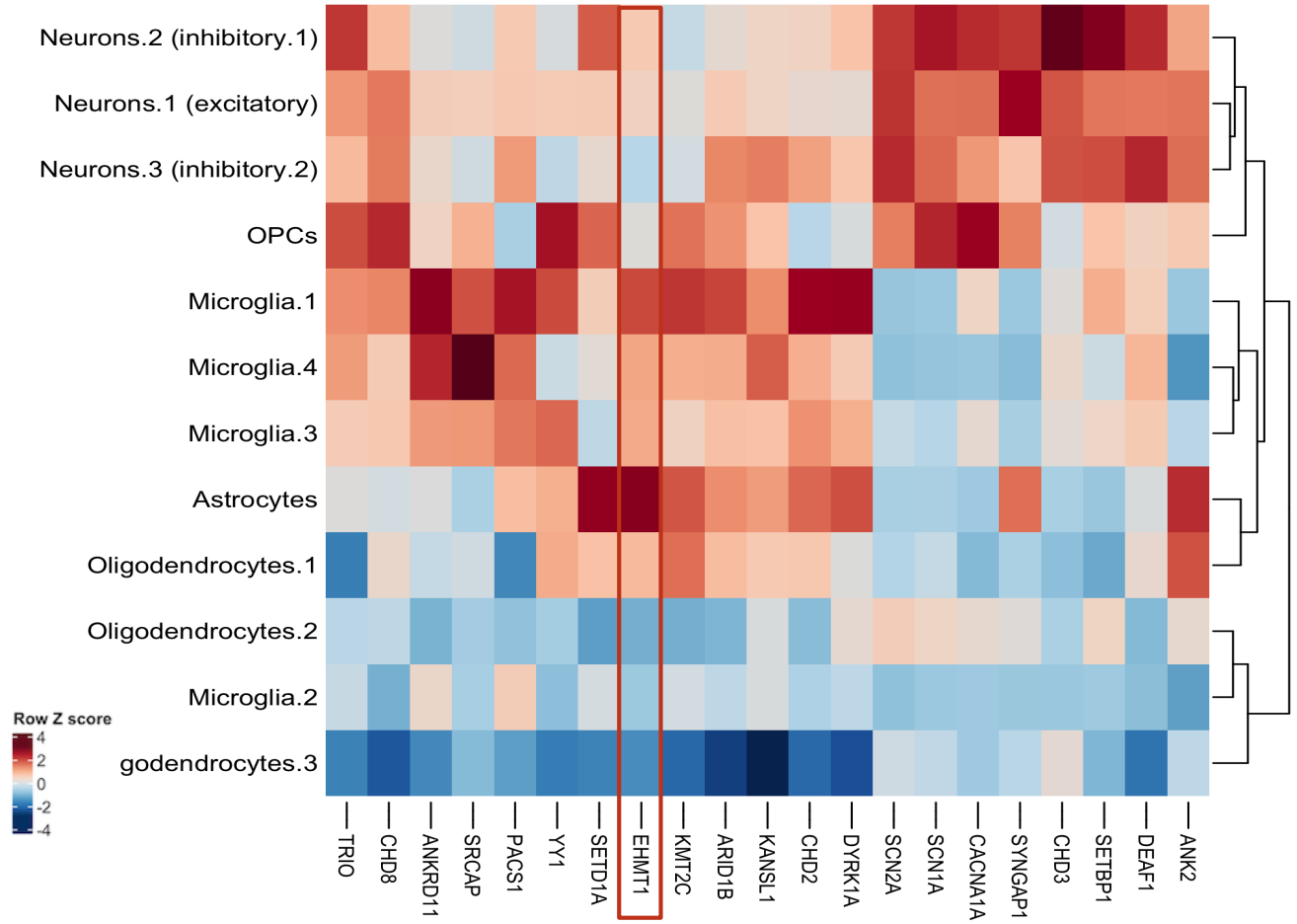
Increased VIP in Amygdala

Relative cell density compared to *Ehmt1*$^{+/+}$

Low High

Negwer M et al, 2021, *Brain struct Funct*
Negwer M et al., *in preparation*

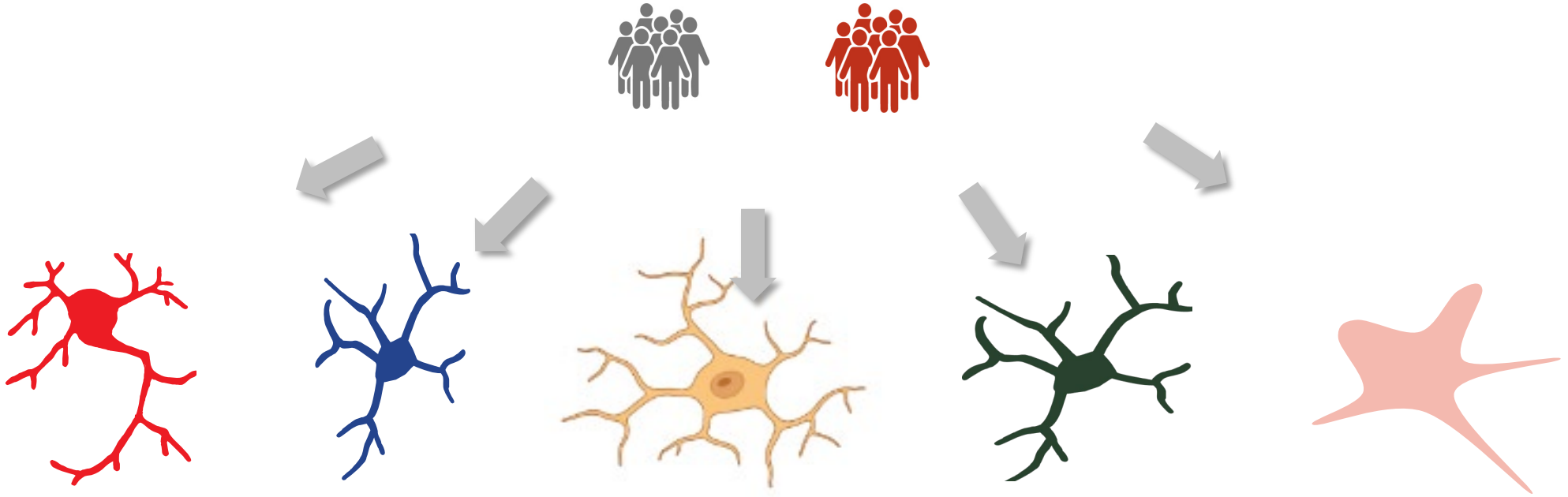
Expression of *EHMT1*

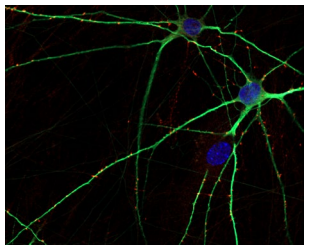
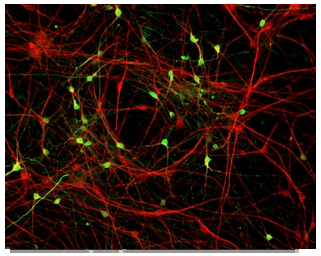
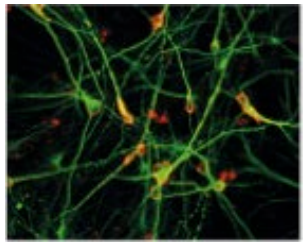
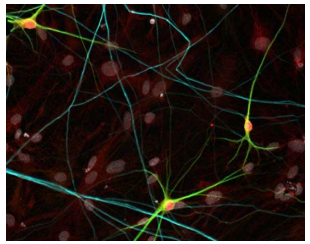
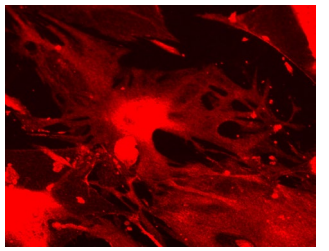


Single-cell data expression post-mortem material

Lot de Wit, *unpublished*

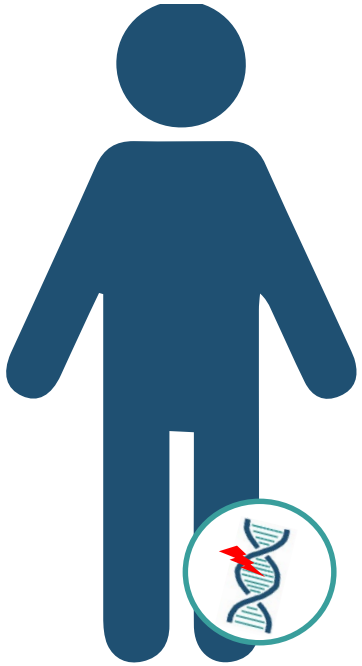
Investigating the cell type-specific function of EHMT1 using iPSC models

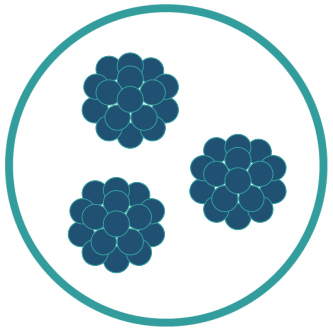


	Glutamatergic neurons	GABAergic neurons	Dopaminergic	Serotonergic	Astrocytes
					
Phenotype	**NMDAR hyperfunction**	**Delayed GABA reversal**	**Minor network effects**	**Minor network effects**	**Increased S100b expression**
Olanzapine response	NO	NO	NO	NO	YES

BRAINmodel

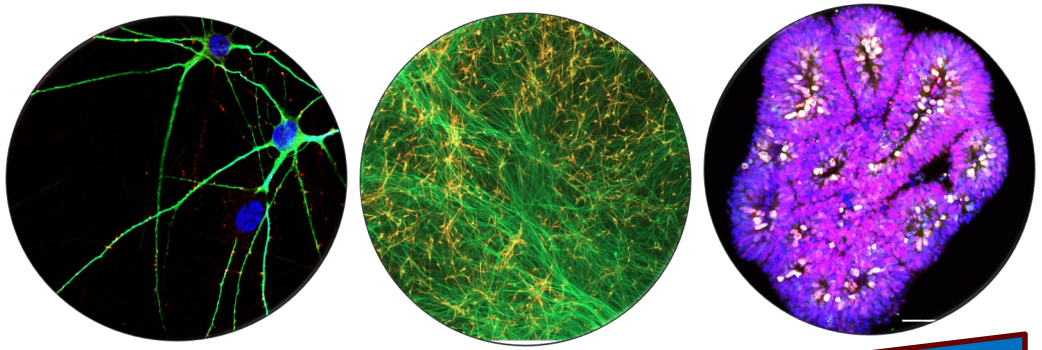
- Development of robust **stem cell-based** 2D and 3D brain models
- Increase <u>robustness</u>, <u>complexity</u>, <u>predictability</u> and <u>scalabilty</u>





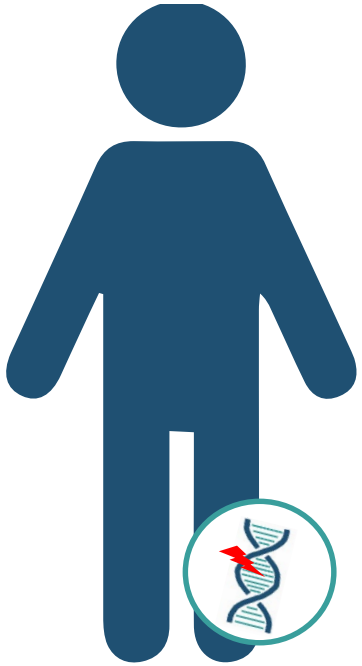
Induced Pluripotent Stem Cells (iPSC)

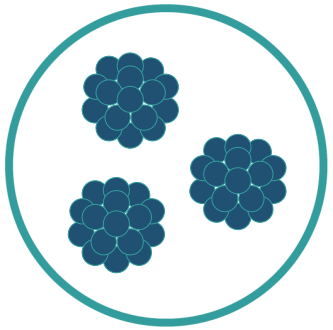
In vitro iPSC derived brain models



BRAINmodel

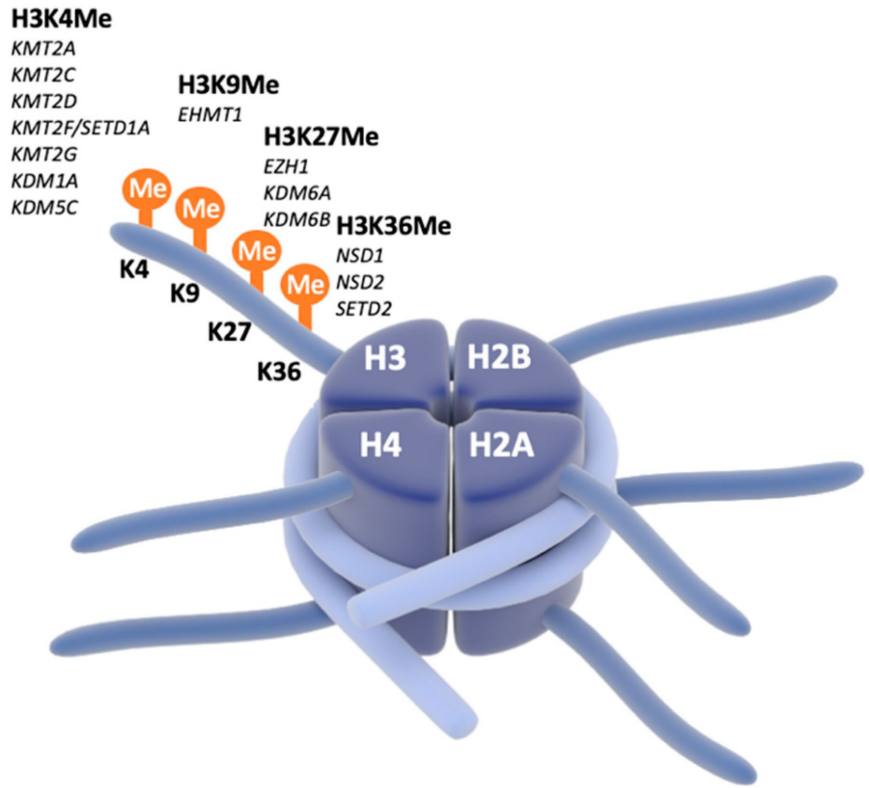
- Development of robust **stem cell-based** 2D and 3D brain models
- Increase robustness, complexity, predictability and scalabilty





Induced Pluripotent Stem Cells (iPSC)

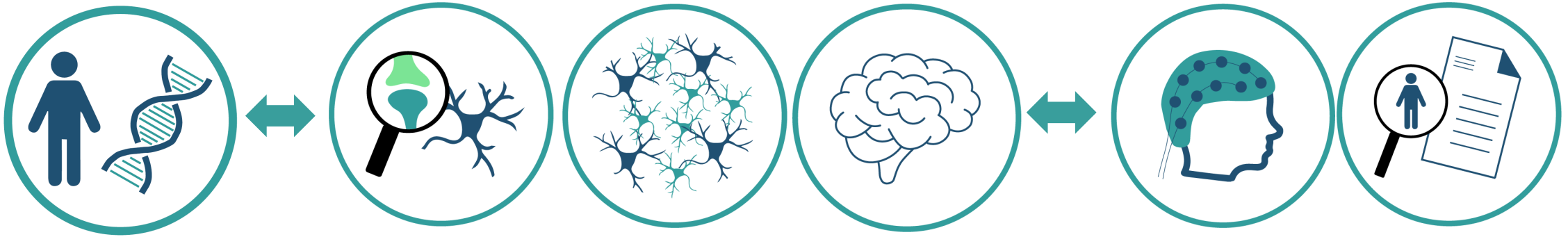
- **Chromatinopathies**
- **Synaptopathies**



–*EHMT1*
–*KMT2A*
–*KMT2D*
–*SETD1A*

BRAINmodel

- Linking **in vitro measurements** to **patient measurements**

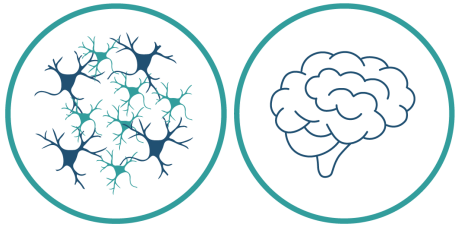


Balance
Excitation-Inhibition

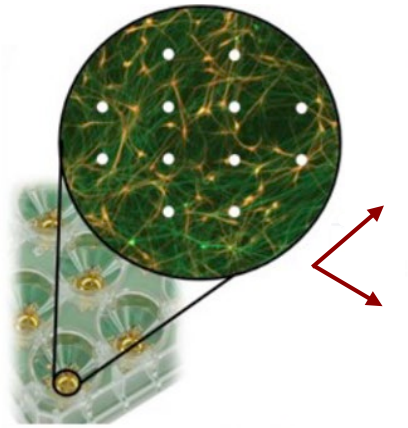
- Based on mechanisms: E/I imbalances - drug reporpusing
- Epigenetic compounds to restore “balance”

BRAINmodel

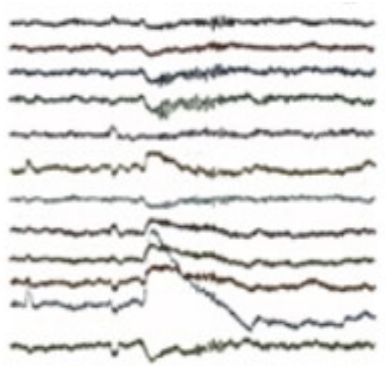
https://brainmodel.nl



Signals from **Brain-on-a-chip** models



Clinical EEG



Biomarkers for prediction

BRAINmodel
UNIVERSITY OF TWENTE.
CLINICAL NEUROPHYSIOLOGY
Nina Doorn
Dr. Monica Frega
Prof. dr. ir. Michel van Putten
https://brainmodel.nl

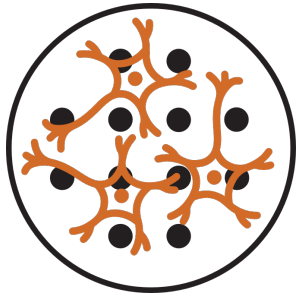
Generate model with data

NDD patient

?

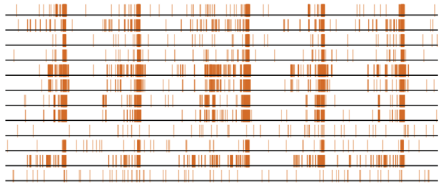
In vitro

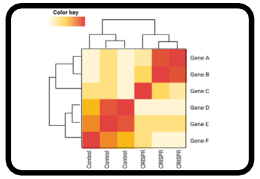
hiPSC derived neuronal networks



Targeted investigation

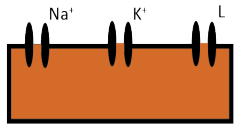
MEA-data



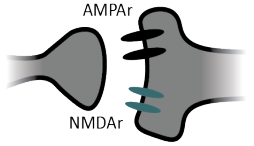


Gene-expression

In silico

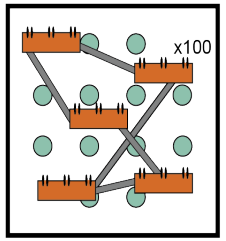


HH neuron model

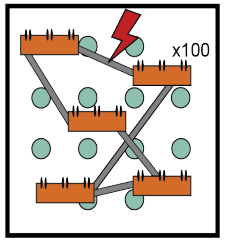


Synapse model

Virtual Electrodes



In silico control model



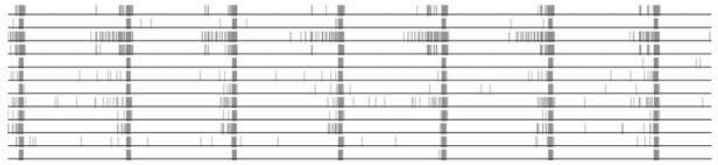
In silico pathological model

patch clamp and MEA data

Discover potent
disease mechani

In Vitro

Basal



In Silico

Basal

Generate predictions about
underlying cellular mechanisms

D. Schubert
H. van Bokhoven
T. Kleefstra

Shan Wang
Eline van Hugte
Katrin Linda
Chantal Schoenmakers
Astrid Oudakker
Ka Man Wu
Britt Mossink
Anouk Verboven
Elly Lewerissa
Ummi Ciptasari
Rachel Mijdam
Naoki Kogo
Brooke Latour
Xiuming Yuan
Emma Dyke
Imke Schuurmans
Nicky Scheefhals
Sofia Puvogel
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D. Koolen
B. Franke

U Twente

M. Frega
M. Van Putten

VU Amsterdam

M. Verhage
N. Cornelissen

SFARI SIMONS FOUNDATION AUTISM RESEARCH INITIATIVE
ZonMw
Epilepsiefonds De Macht van het Kleine
EUROPEAN JOINT PROGRAMME RARE DISEASES
NWO
E-Rare
neuron Network of European
BRAINmodel

Summary

Neuronal Network

Hyperactivity

Reduced network organization

Cellular

Decreased glutamate uptake
Altered Ca^{2+} signalling
Neuroinflammation

Molecular

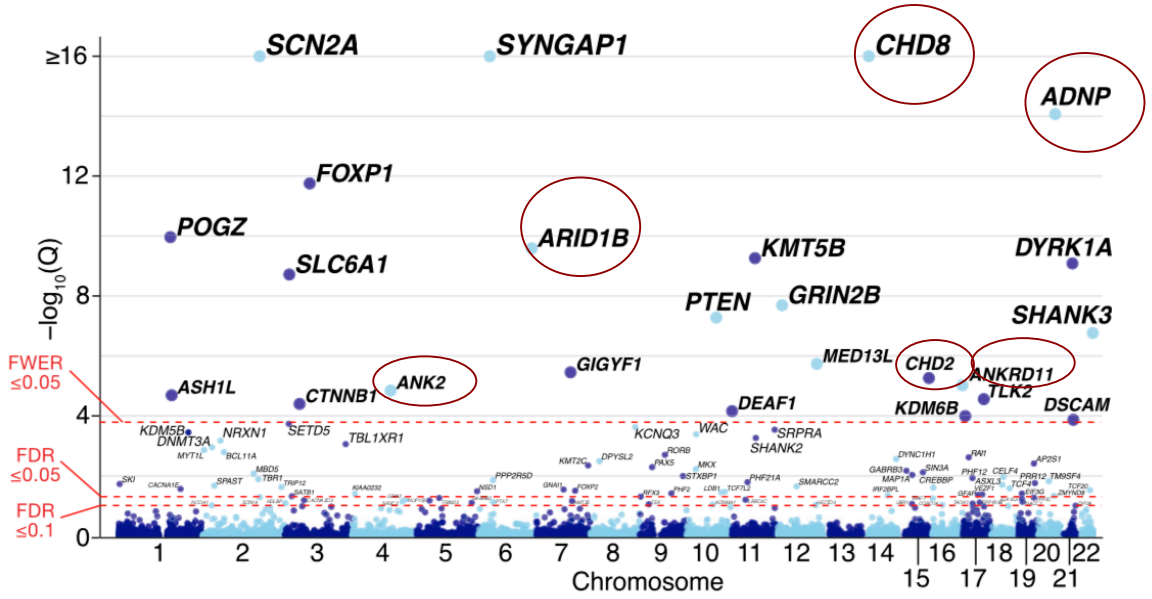
Increased *S100B* expression and decreased H3K9me2 at *S100B* loci

Rescue

Rescue with S100B antagonist **olanzapine**

Wang S. et al., in preparation

Can we identify disease/patient-specific neuronal network phenotypes?

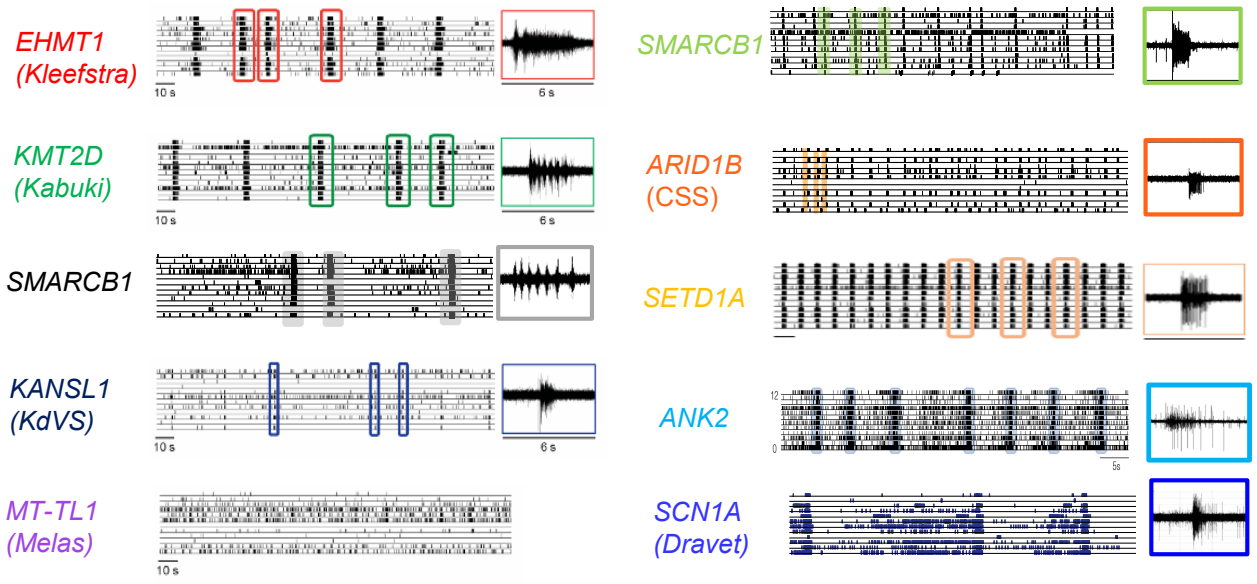


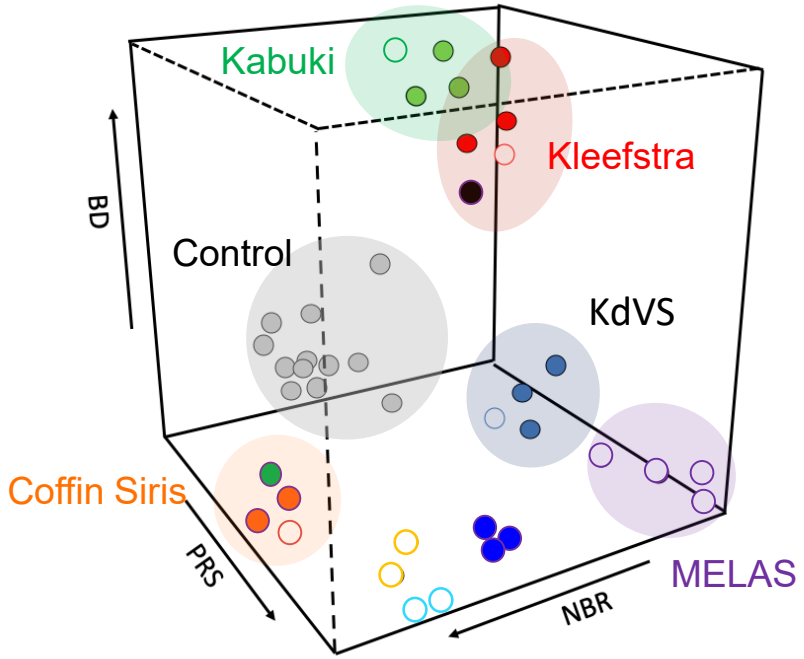
Satterstrom et al., *2020 Cell*

EHMT1
KMT2C
SMARCB1
MBD5
ARID1A
ARID1B
SETD1A
KANSL1
CHD2
CHD3
CHD8

ANK2
SCN1A
CACNA1A
MELAS
NANS
PMM2
CDH13
PCDH19
ADNP
ANKRD11
YY1

Syndrome-specific neuronal network phenotypes



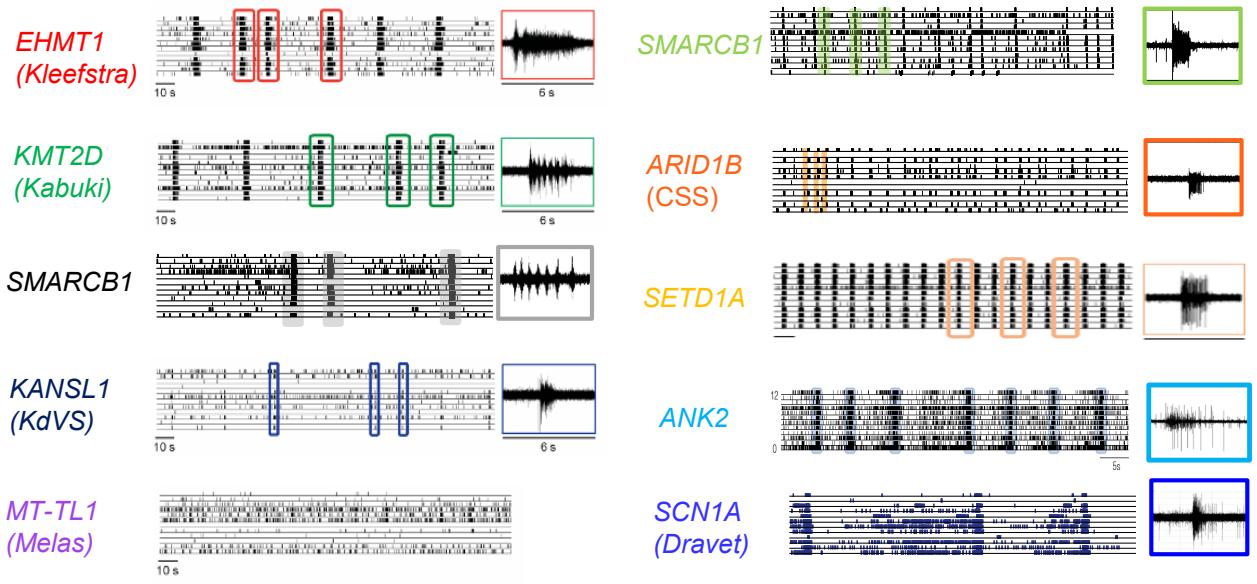


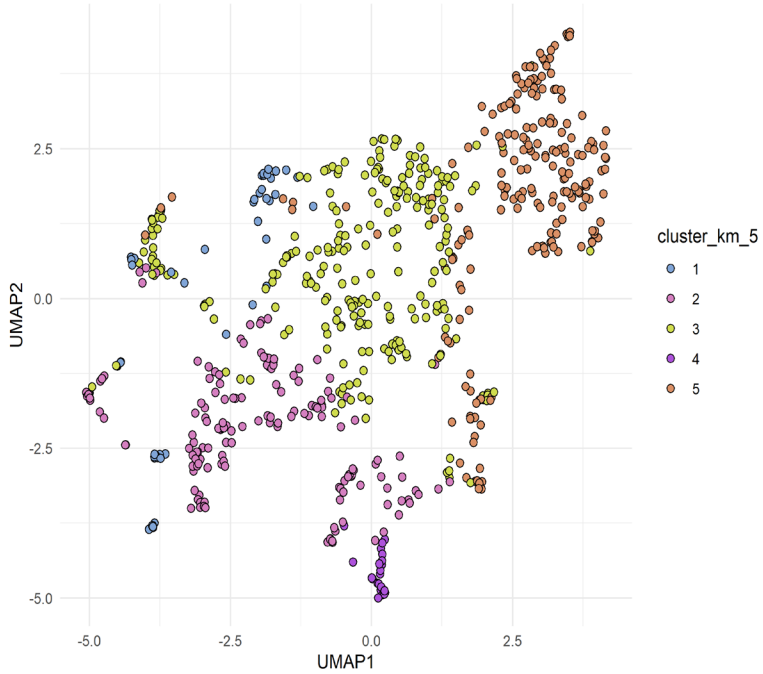
Closed circles show independent IPSC lines
Open circles show CRISPR/Cas9 isogenic lines

- 15 syndromes
- 1-5 cell lines per disease
- 39 cell lines
- >1000 recordings

Frega et al., Nat Comms 2019
Klein Gunnewiek et al., Cell Rep 2020
Frega et al.,, Cell Rep 2020
Frega et al., Cell Rep 2020
Mossink et al., Stem Cell Rep 2021
Mossink et , Mol Psych 2021
Linda et al., Autophagy 2021
Shan et al., Cell Rep 2022
Gerosa et al., Cell Rep 2022

Syndrome-specific neuronal network phenotypes





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