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patient-based drug discovery

A patient-derived disease model of Phelan McDermid Syndrome for the identification of SHANK3-specific compounds

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How to create a neuronal disease model and screen compounds with it

- Most neurological disease are hard to treat

  Occurrence **early in life**: Development → *Treatment possible?*

  Occurrence **throughout life**, but detection late → *Treatment possible?*

- Rare neurological disease are useful for mechanistic studies & drug development: Often a mono genetic "in-road" or "trigger" is known

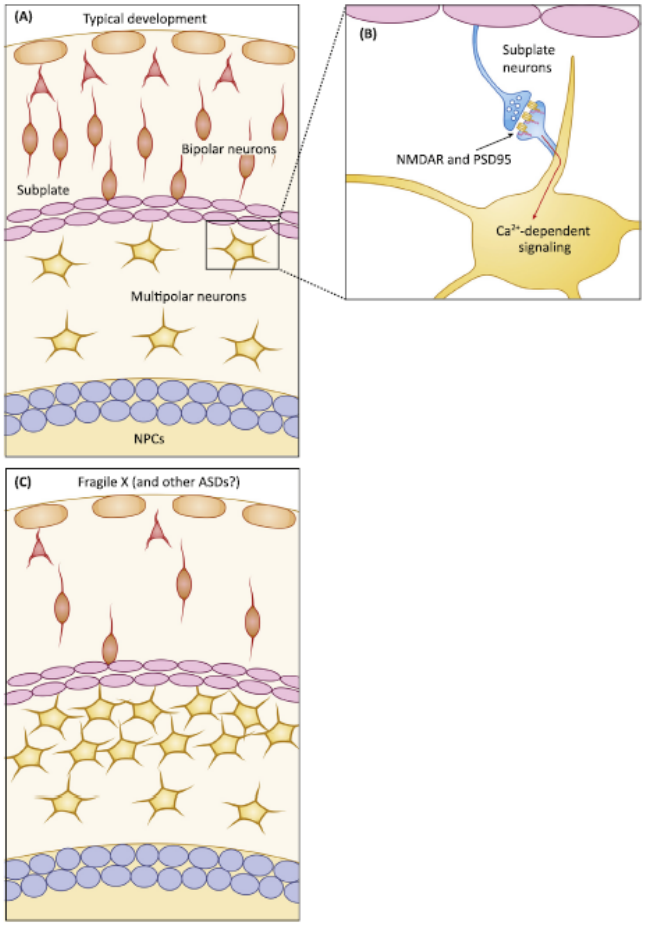
- Can rare/genetic forms of a disease serve as an example for a general class of diseases?

  Parkinson's disease: Protein aggregation, mitochondrial defects, signaling pathways (LRRK2)

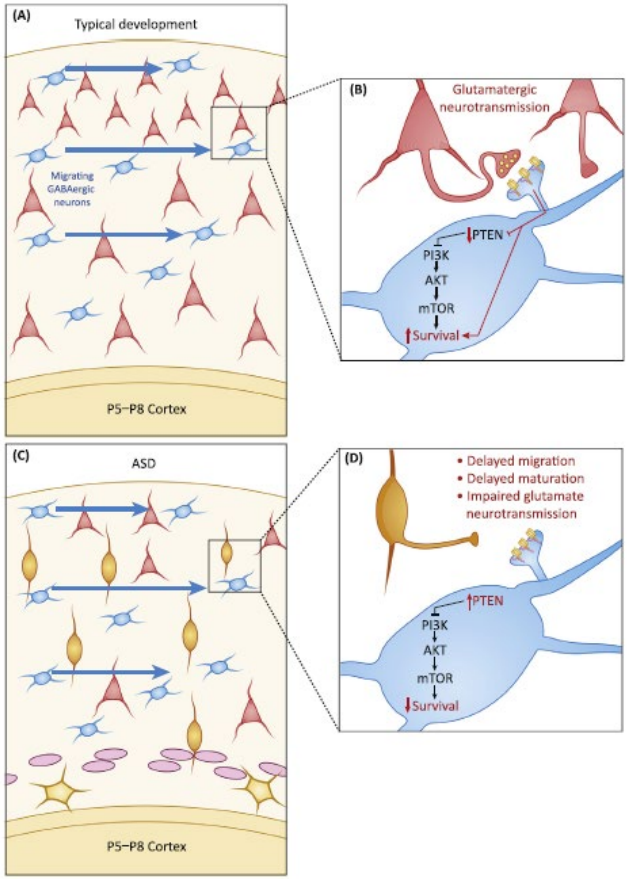
  Autism spectrum: Neuronal development, synaptic biology, network activity modulation (stim/inh)

ASDs have an interconnected Developmental & Synaptic pathophysiology

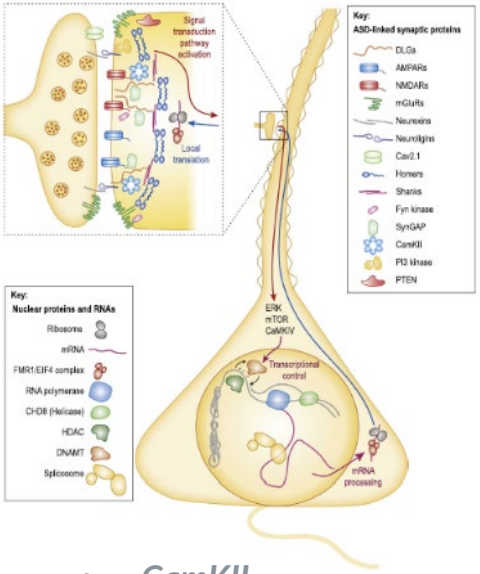
1. Glutamatergic Neurotransmission is Required for Cortical Migration



2. Glutamatergic Neurotransmission is Required for Neuron Survival



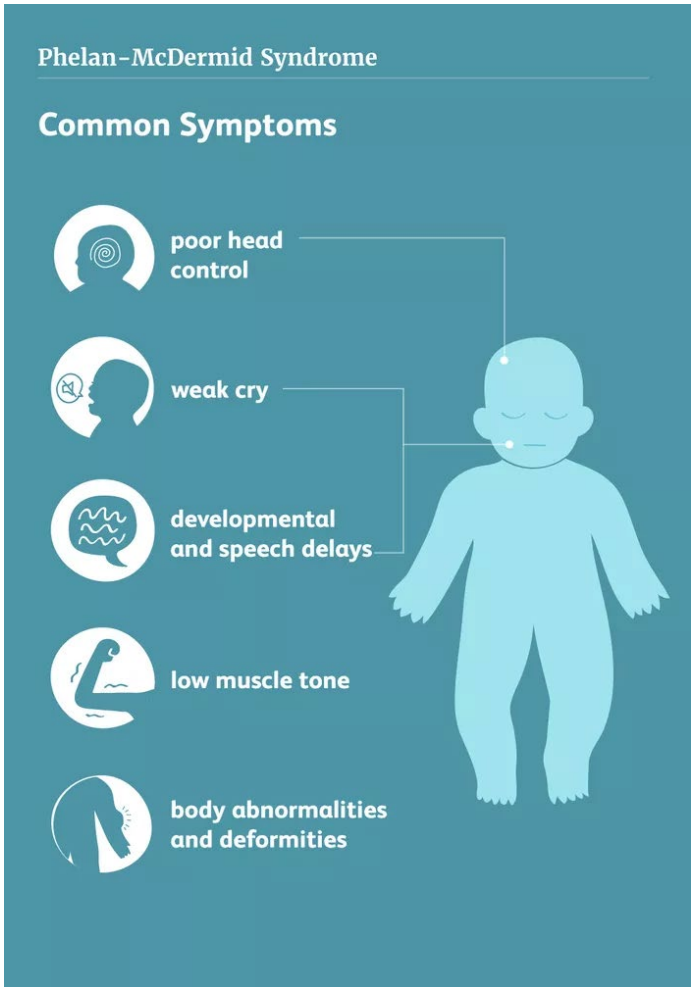
3. Developmental Signal Transduction Cascades Influence Synaptic Plasticity



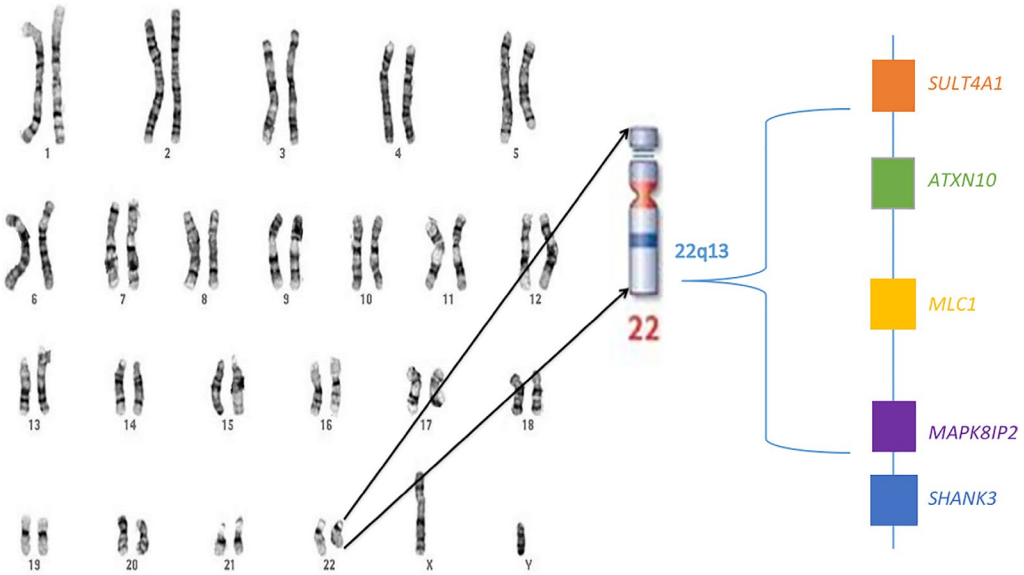
- *CamKII*
- *PI3 kinase*
- *PTEN*
- *HDACs*

Heavner *et al.*, Trends Neurosci, 2020

Phelan-McDermid syndrome (PMS)



- Part of the autism spectrum disorders
- PMS is caused by deletions/alterations on chromosome 22
- SHANK3 always impacted



Ziats *et al.*, PLoS One, 2019

SHANK3$^{+/-}$ hiPSC neurons can model Phelan–McDermid syndrome (PMDS)

Strategy 1: Elevate SHANK3 expression

- Reduced SHANK3 expression contributes to synaptic defects in PMDS neurons

Research Paper

Human Pluripotent Stem Cell-derived Cortical Neurons for High Throughput Medication Screening in Autism: A Proof of Concept Study in SHANK3 Haploinsufficiency Syndrome

Hélène Darville [a], Aurélie Poulet [a], Frédérique Rodet-Amsellem [b], Laure Chatrousse [a], Julie Pernelle [c,d], Claire Boissart [a], Delphine Héron [e,f], Caroline Nava [e,f], Anselme Perrier [c,d], Margot Jarrige [c,d], Francis Cogé [g], Mark J. Millan [g], Thomas Bourgeron [h,ij], Marc Peschanski [c,d], Richard Delorme [b,h], Alexandra Benchoua [a,*]

- Rescue is possible by LiCl and has positive clinical outcome; study ongoing

NIH U.S. National Library of Medicine

ClinicalTrials.gov

Effect of Lithium in Patients With Autism Spectrum Disorder and Phelan-McDermid Syndrome (SHANK3 Haploinsufficiency) (Lisphem)

ClinicalTrials.gov Identifier: NCT04623398

Strategy 2: SHANK3-independent approach

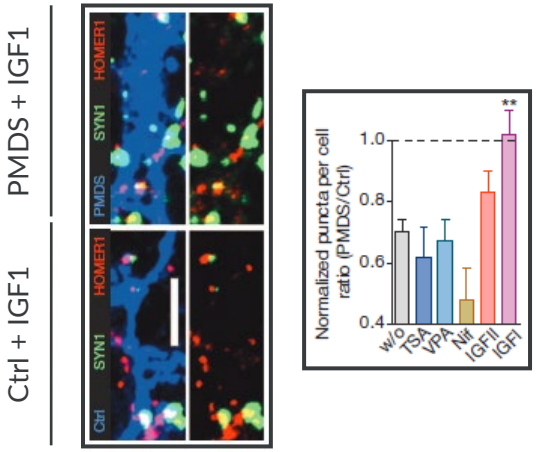
- PMDS: reduced expression of glutamate receptors and decreased number of synapses (excitatory/inhibitory balance)

LETTER

doi:10.1038/nature12618

SHANK3 and IGF1 restore synaptic deficits in neurons from 22q13 deletion syndrome patients

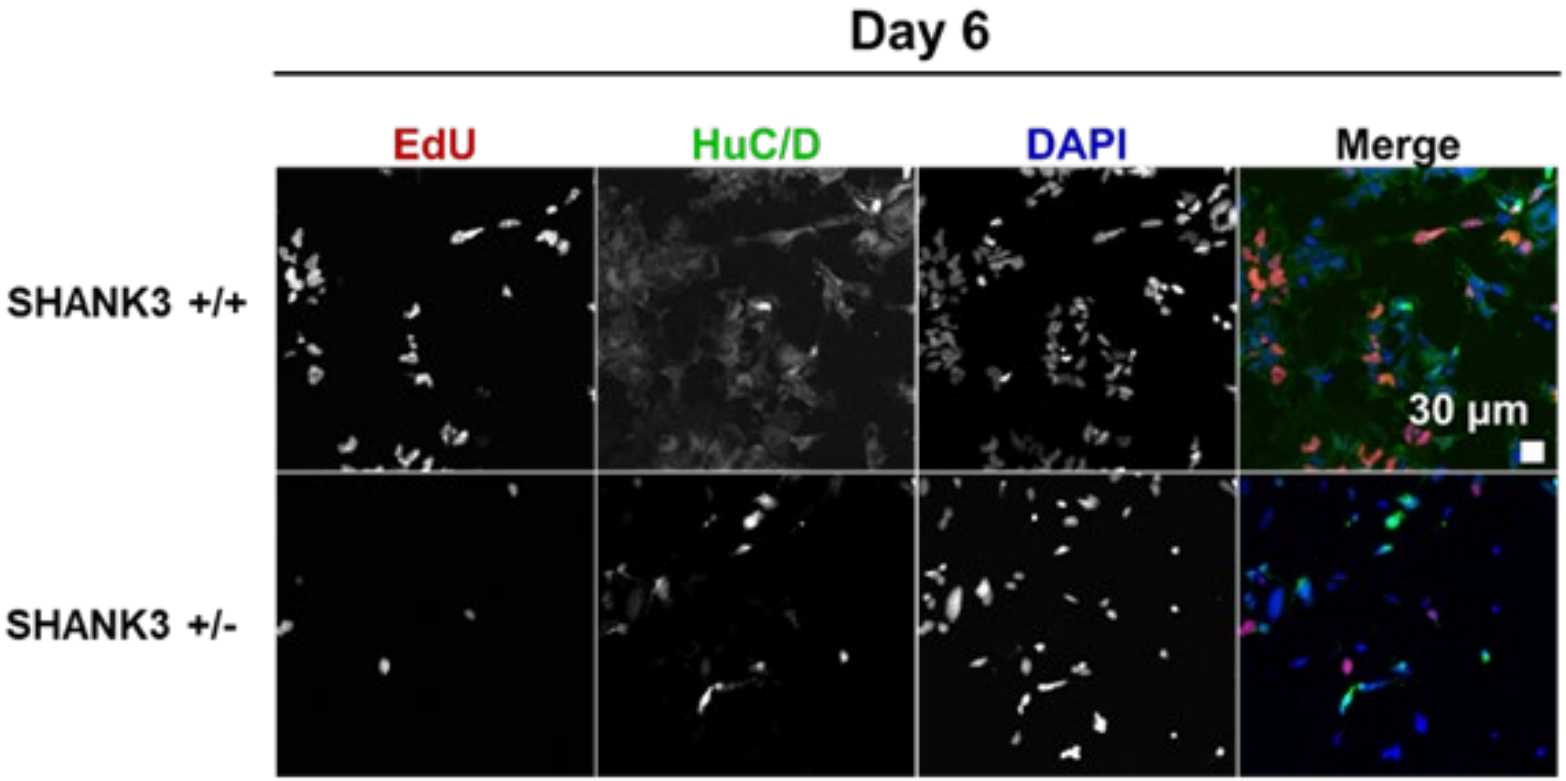
Aleksandr Shcheglovitov[1], Olesya Shcheglovitova[1], Masayuki Yazawa[1], Thomas Portmann[1], Rui Shu[1], Vittorio Sebastiano[2,3], Anna Krawisz[1], Wendy Froehlich[4,5], Jonathan A. Bernstein[4], Joachim F. Hallmayer[5] & Ricardo E. Dolmetsch[6]



- IGF1: SHANK3-independent rescue possible
- Alternative drugable pathways?

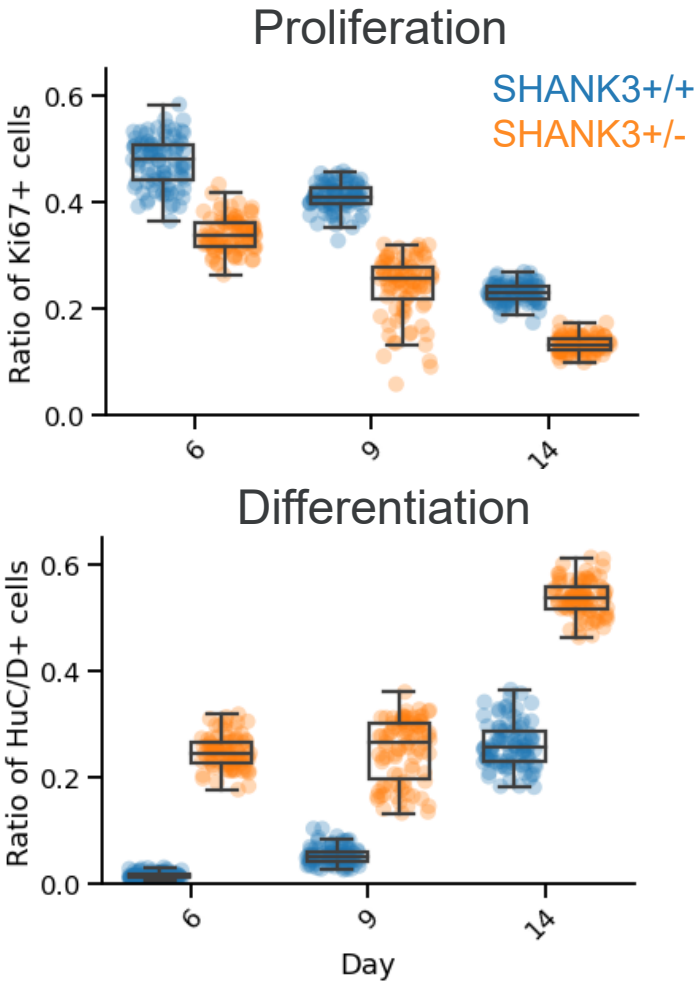
Darville *et al.*, EBioMedicine, 2016
Shcheglovitov *et al.*, Nature, 2013

SHANK3+/- haploinsufficient NPCs differentiate faster *and proliferate slower*

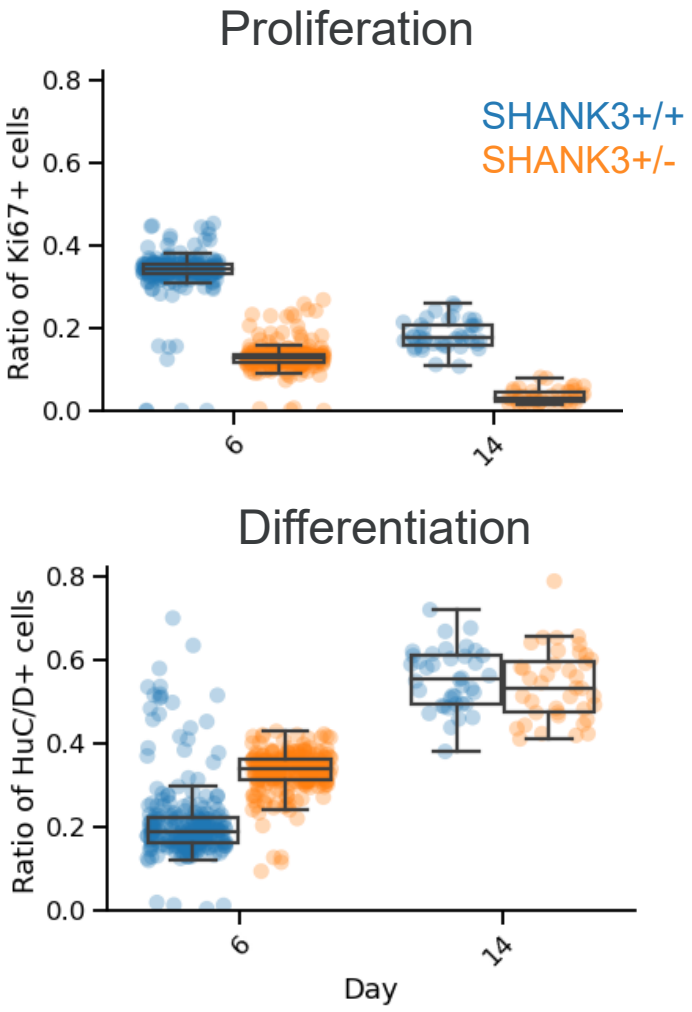


SHANK3+/- haploinsufficient NPCs differentiate faster

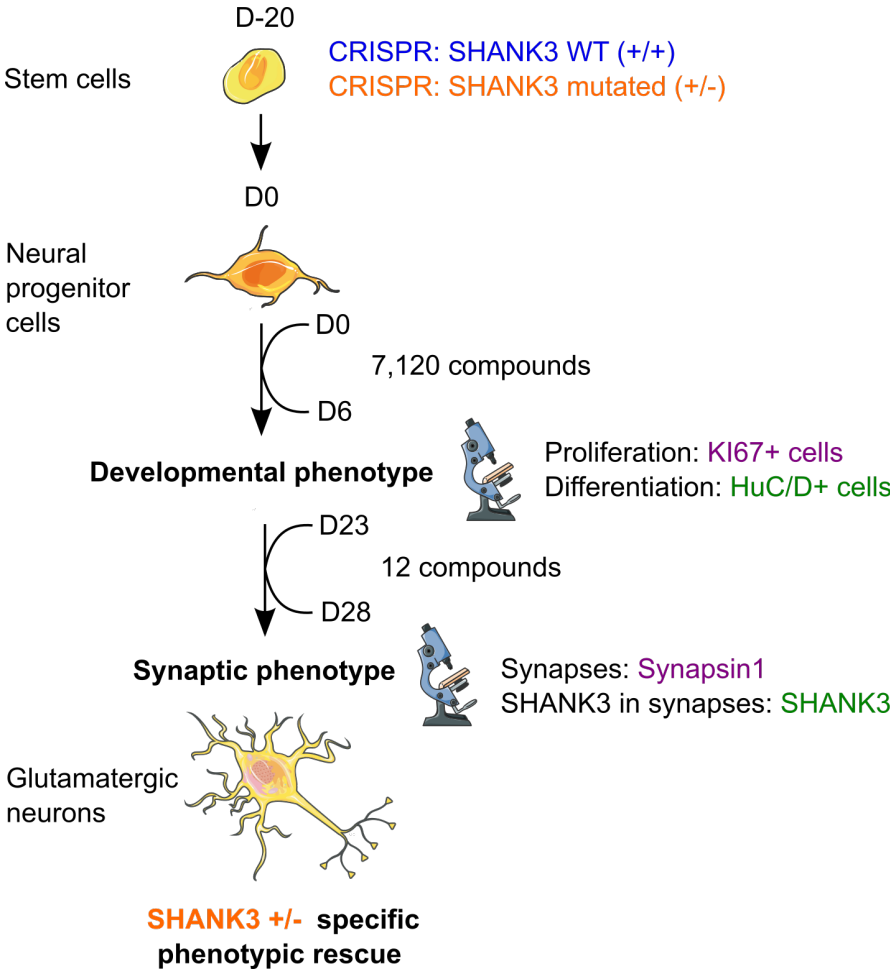
Patient-derived NPCs



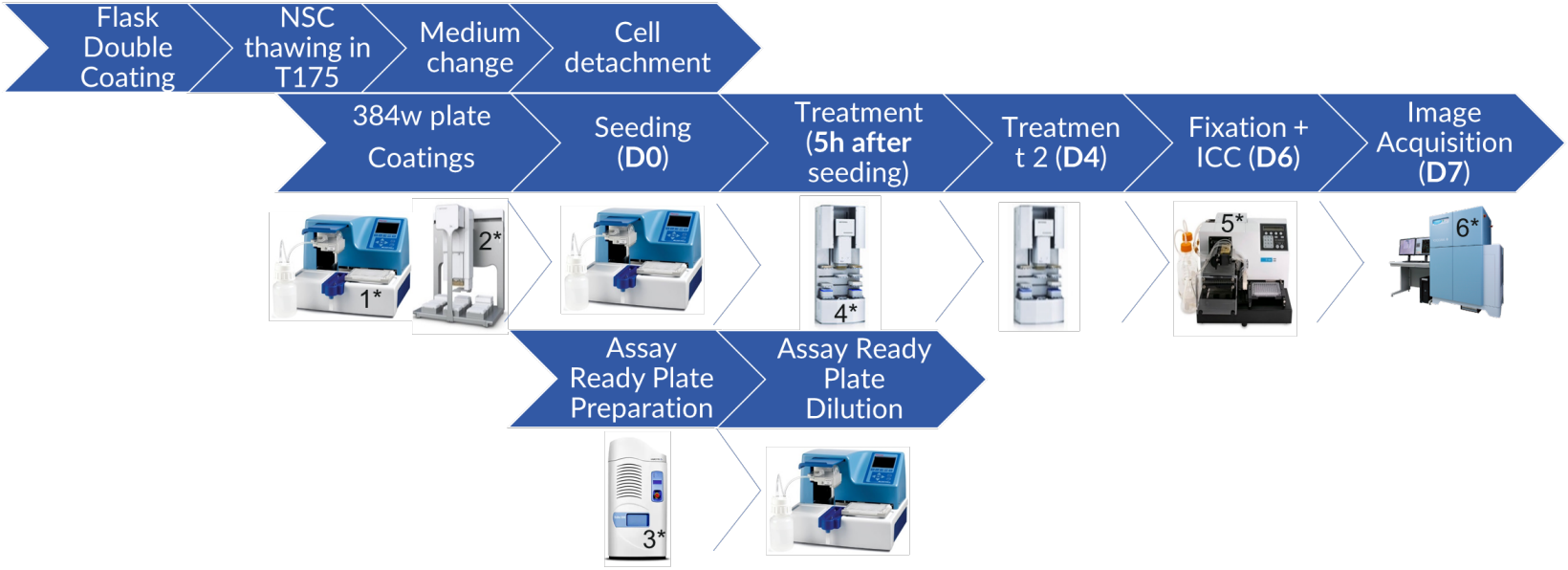
CRISPR-engineerd NPCs



Step 1: Overall screening strategy design

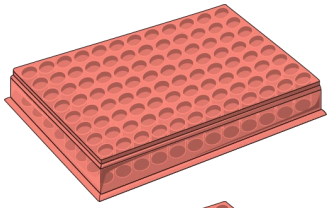


Step 2: Cell handling & protocol automation



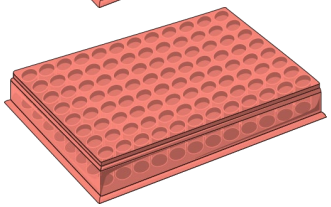
1* ThermoFisher Multidrop Combi; 2* Agilent Bravo; 3* Labcyte Echo555, 4* Agilent Vprep; 5* Biotek EL406; 6* Yokogawa CV7000

Overview: Screening of 7,560-compound MoA library

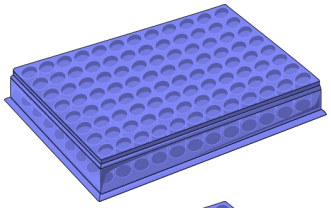


Rep 1

HT
(SHANK3+/-)

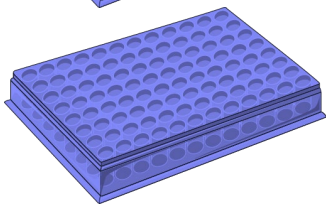


Rep 2



Rep 1

WT
(SHANK3+/+)



Rep 2

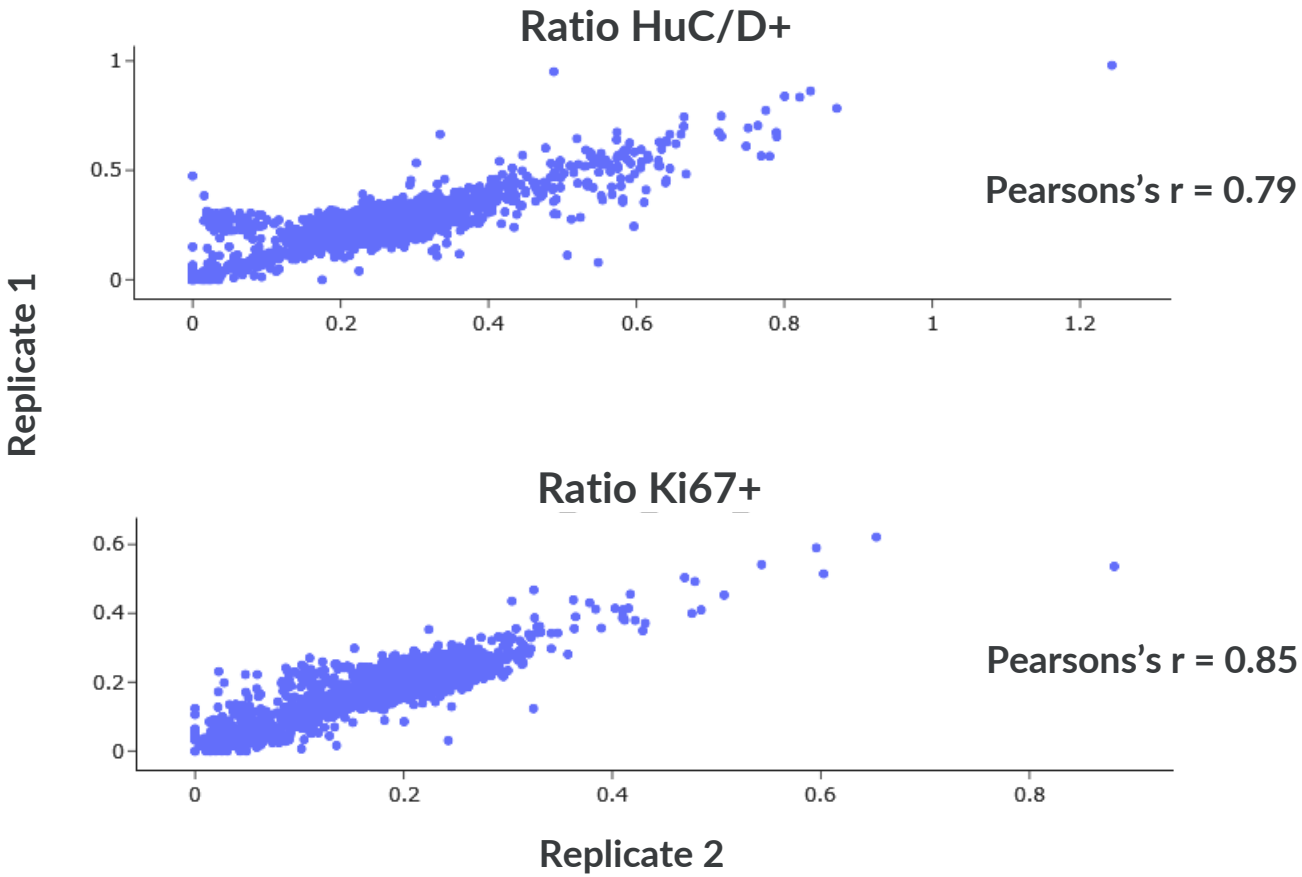
1 µM

6 days

Desired hit profile

	HT	WT
Proliferation (Ki67+ nuclei)	↑	=
Differentiation (HuC/D+ nuclei)	↓	=

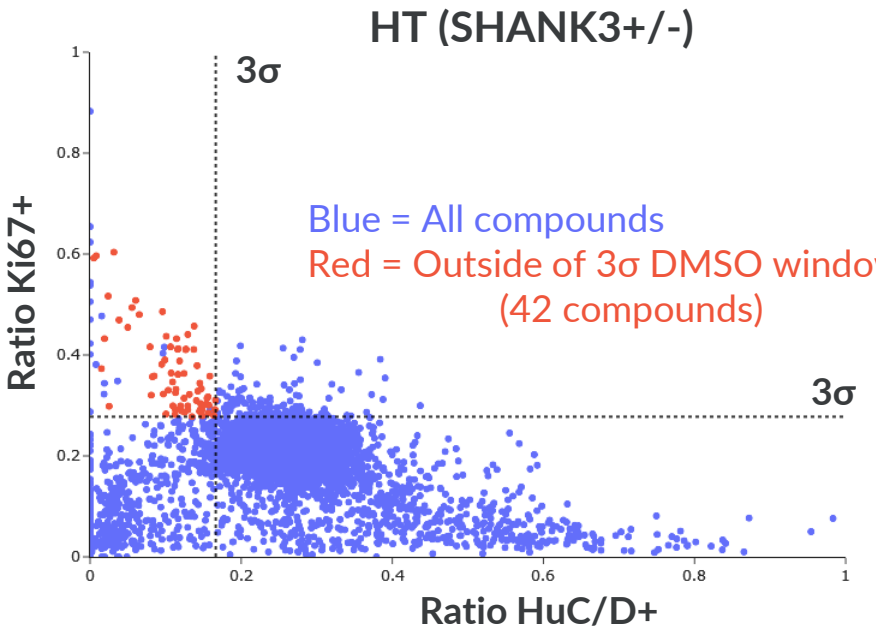
Step 3: Primary screening
The screening protocol is technically robust between replicates

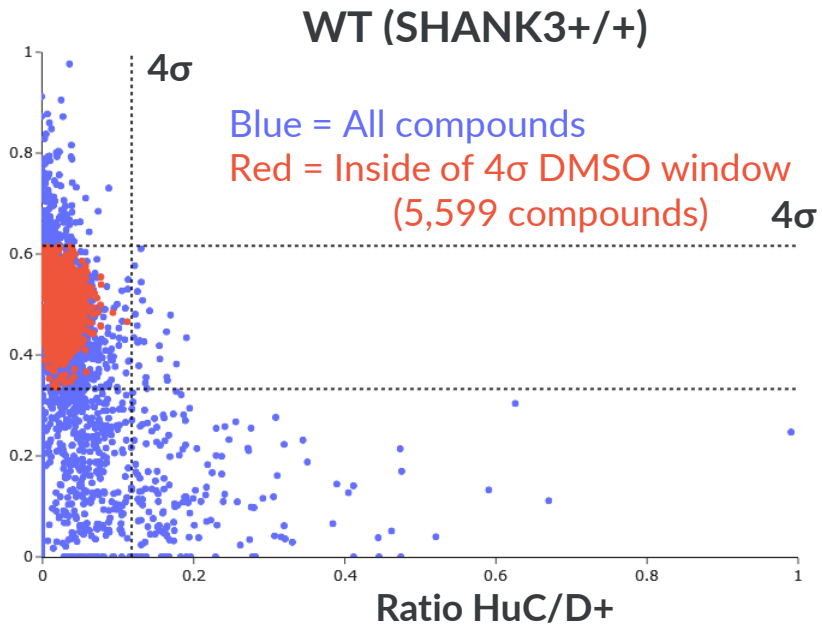


HT (SHANK3+/-) data shown only.

Step 3: Primary screening

22 molecules decrease differentiation specifically in SHANK3+/- NPCs



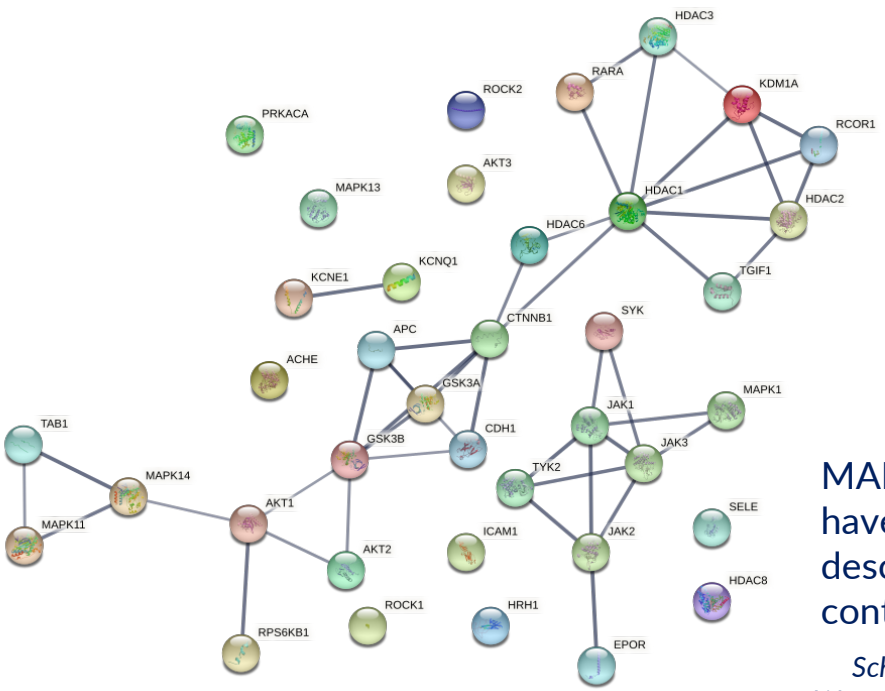


- HT (SHANK3+/-) specific increase in Ki67 proliferation and decrease in HuC/D differentiation
- **Desired hit profile: Outside of HT 3σ window, but inside of WT 4σ window = 22 compounds**

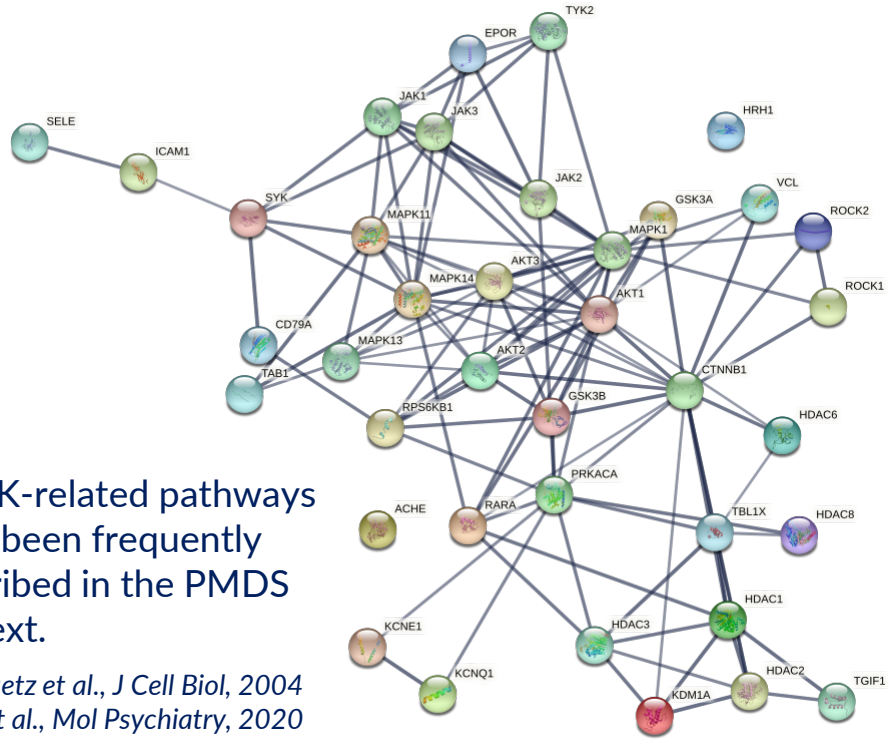
Known compound MoAs allow functional clustering

22 hits: Multiple targets related to β-catenin & AKT/MAPK pathways

Physical association
(thickness = confidence)



Functional and physical association
(thickness = confidence)



MAPK-related pathways have been frequently described in the PMDS context.

Schuetz et al., J Cell Biol, 2004
Wang et al., Mol Psychiatry, 2020
Ingiosi et al., eLife, 2020
Torossian et al., Neurobiol Dis, 2021
Mossa et al., Mol Psychiatry, 2021

STRING

string-db.org
https://version-11-5.string-db.org/cgi/network?networkId=bQPWXIwwuMwJ

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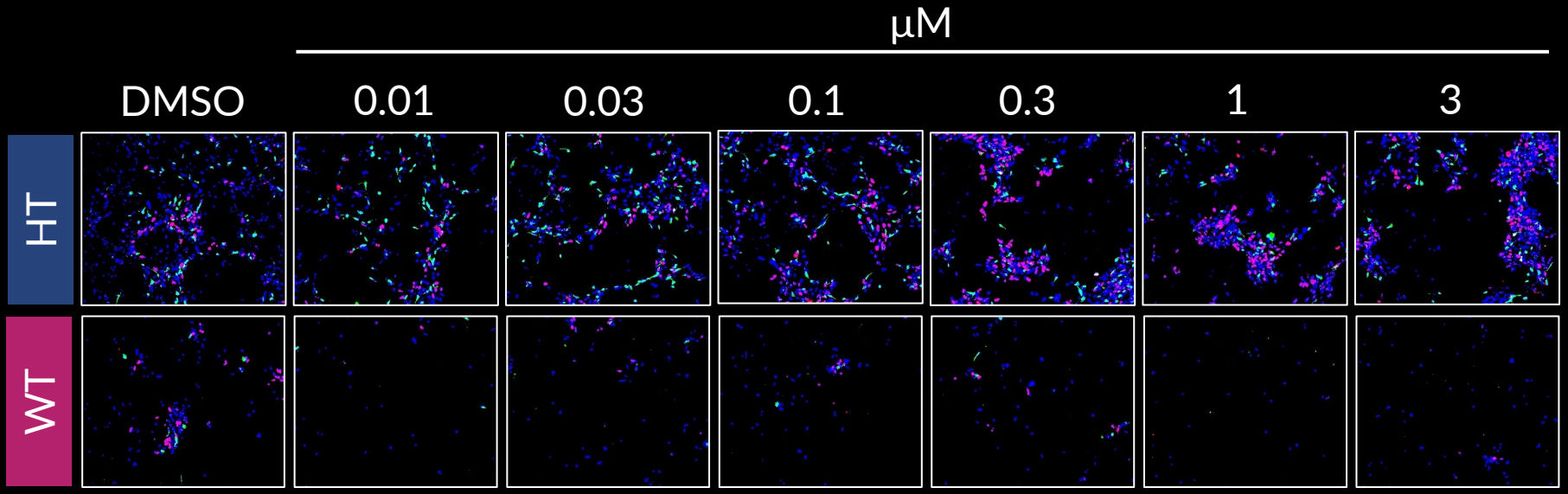
Step 4: Chemical and biological hit validation

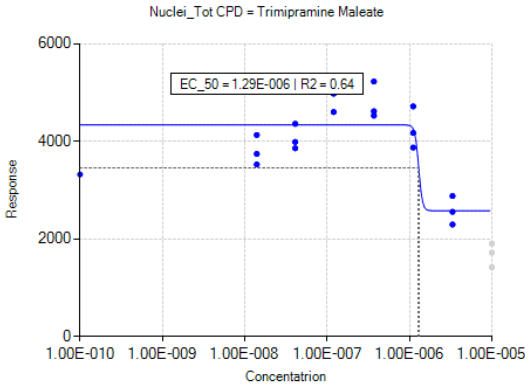
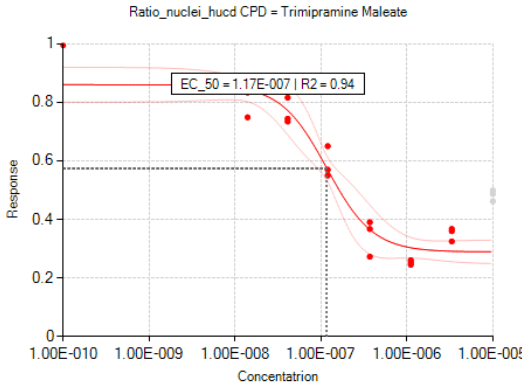
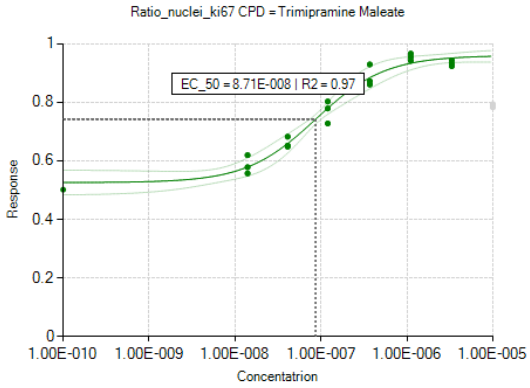
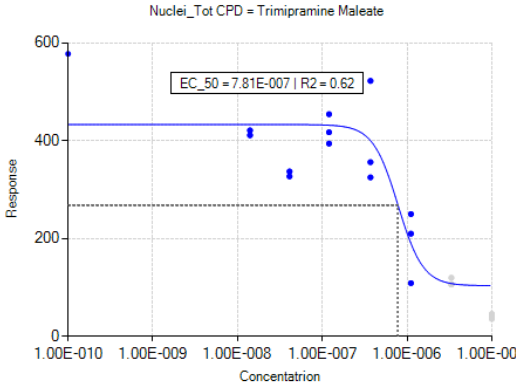
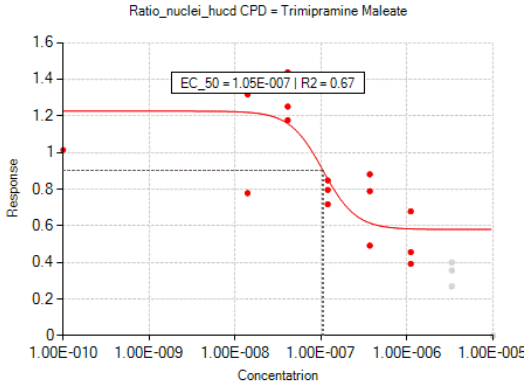
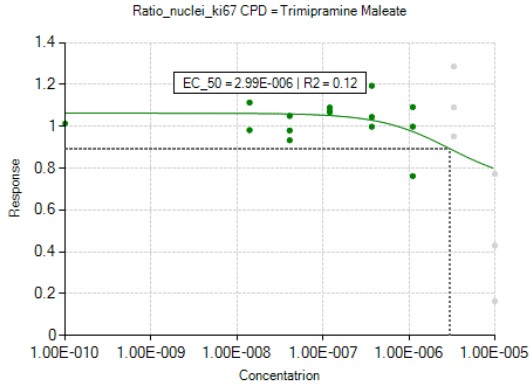
- Hit list expansion (i.e. addition of “toxic” molecules with similar target)
- Testing of independent compound batches
- Compound evaluation on different genetic background (patient-derived cells)

Step 4:
Compound #11 – Validation in original patient derived NPCs



10 µM image not shown.

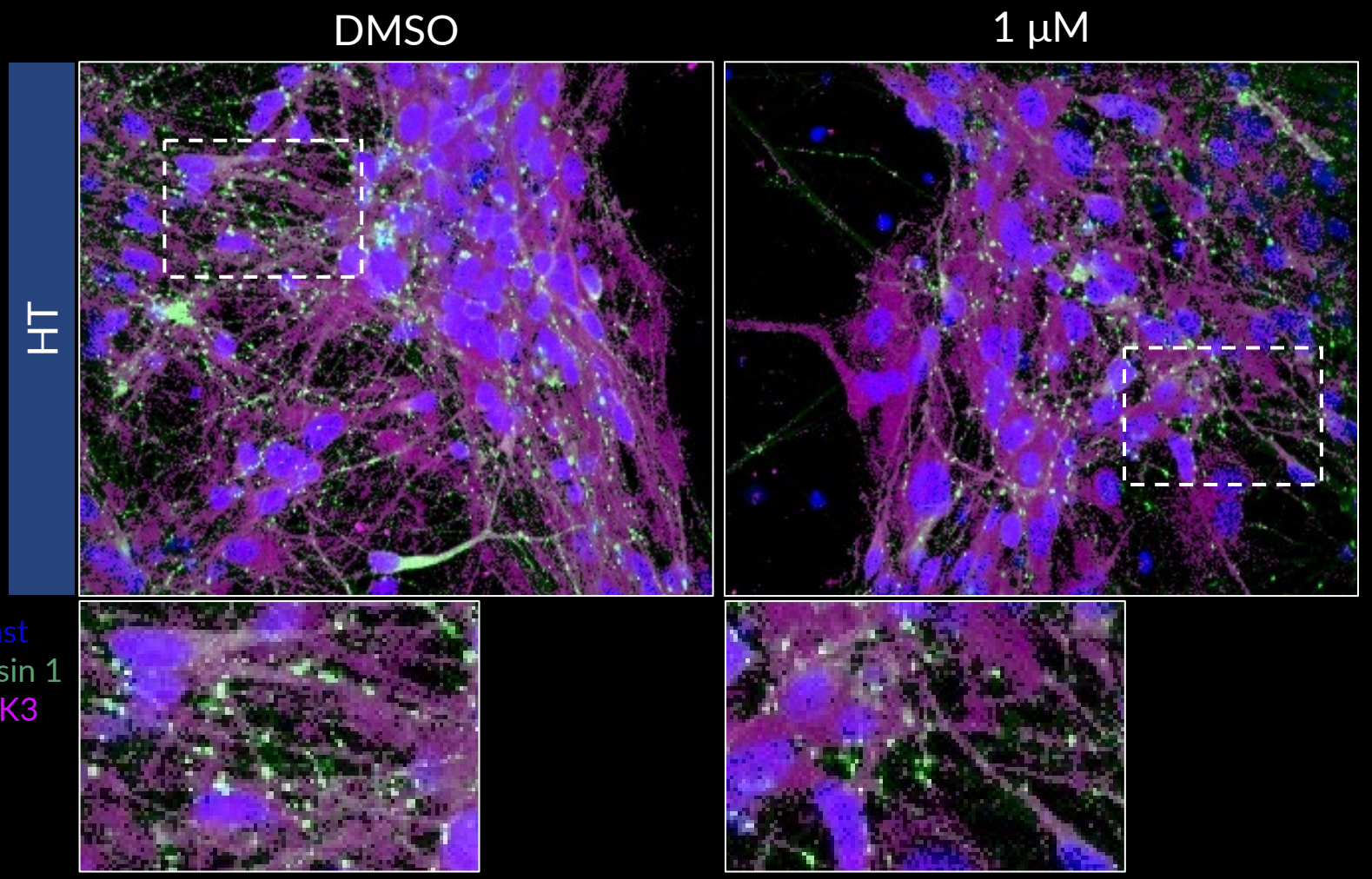
Step 4:
Compound #11 – Validation in original patient derived NPCs

	Nuclei	Differentiation	Proliferation
HT			
WT			

Step 5: Secondary screening
Reversal of hyperdifferentiation-linked increase in synapses

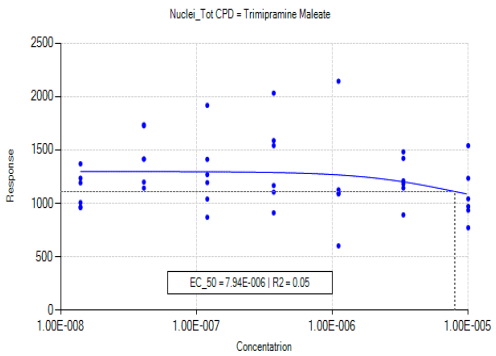
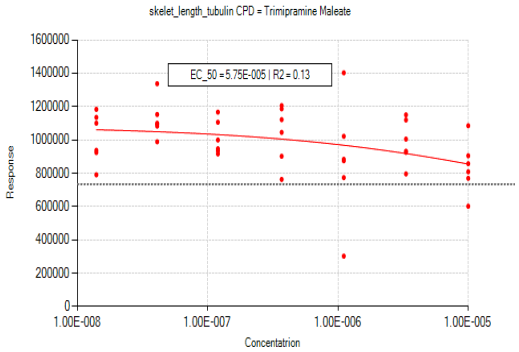
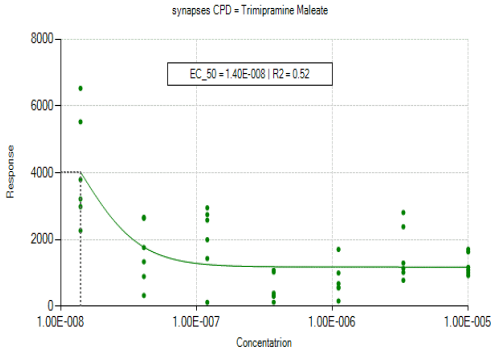
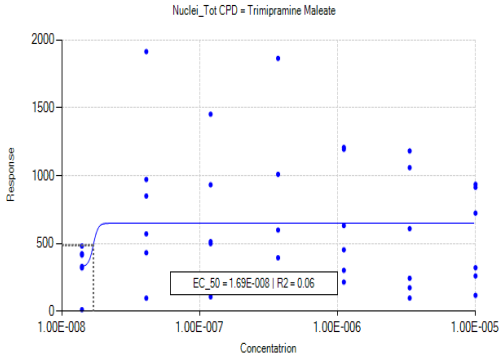
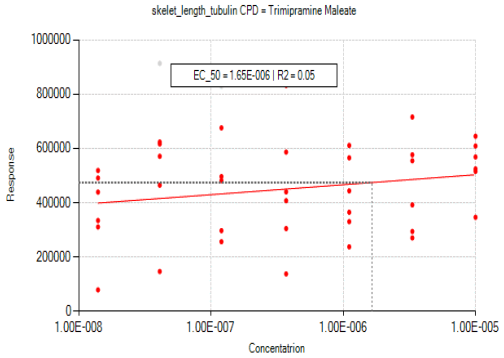
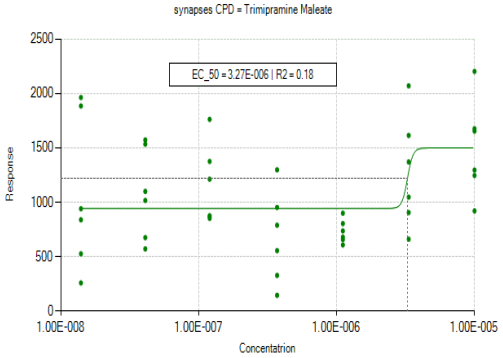
- Focus on D28 synaptic phenotype (compound treatment D21-D28)
- Previously identified phenotype: SHANK3+/- dependent hyperdifferentiation leading to increased numbers of synapses.
- Analysis endpoints:
  1. Dose dependent decrease of **MAP2**/**SHANK3**/**Synapsin** triple positive punctae (“synapses”).
  2. No dose dependent impact on dendtritic MAP2 network.
  3. No strong signs of toxicity (nuclear count).
  4. SHANK3+/- specificity in ESC- and iPSC-derived neurons.

Step 5: Secondary screening
Compound #11 – Reduction of synaptic punctae in CRISPR NPCs



- Decreased number of synapses
- Similar neuronal network length
- Similar expression level of SHANK3

Step 5: Secondary screening
Compound #11 – Reduction of synaptic punctae in CRISPR NPCs

	Nuclei	Network length	Synapses
HT			
WT			

Take home messages

- Mutations in SHANK3 cause Phelan-McDermid Syndrome (PMDS)
- PMDS falls into the autism spectrum and is linked with macroencephaly, a form of neuronal hyperdifferentiation
- Neuro(developmental) disease models are amendable to chemical genomics
- Multiple SHANK3+/- specific compounds slow neuronal differentiation observed at two different timepoints using two different experimental assays
- When added later during the differentiation, two compounds also reduce the number of synapses without impacting the neurite network

Environment, genes and neurological disease

- We won't be able to study climate change-effects in stem cell derived neurons
- But: We might be able to identify chemical modifiers of targets or pathways related to stressors or external effects identified by other experimental/data mining approaches.
- Keep in mind: Chemical genetics approach presented here today can be applied to key pathways of interest.

Thank you for your attention!

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→ Automation & screening

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→ Data analysis

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THANK YOU!

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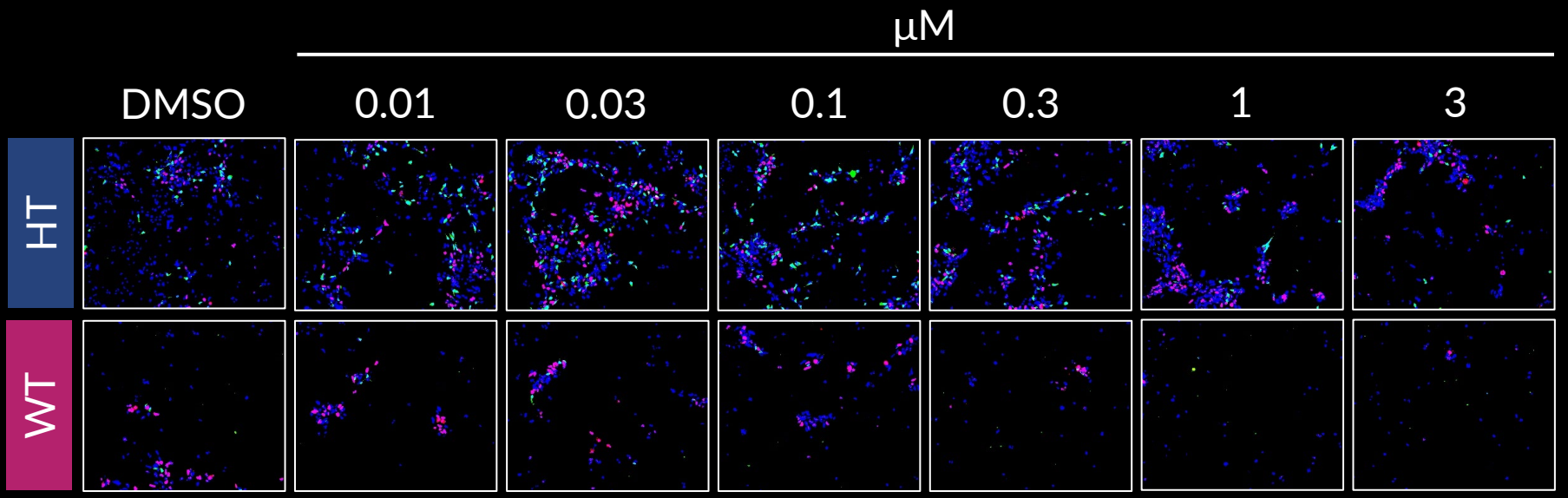
Universität Heidelberg
Université de Strasbourg
Zentral Institut Mannheim

Step 4:
Compound #11 – Validation in original CRISPR NPCs



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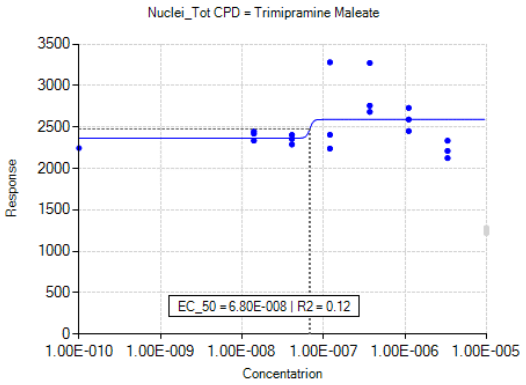
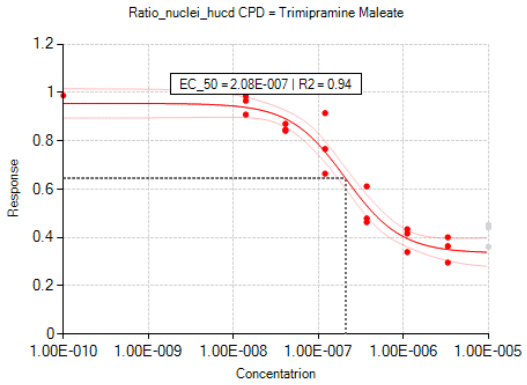
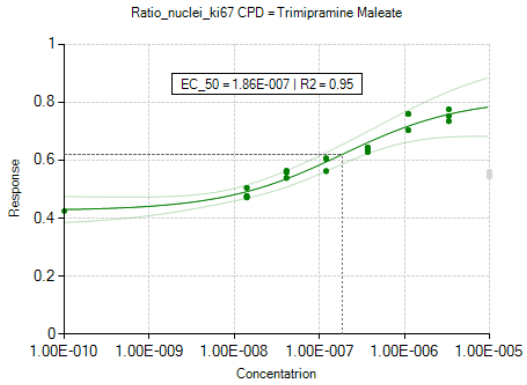
Compound #11 – Validation in original CRISPR NPCs

	Nuclei	Differentiation	Proliferation
HT			
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