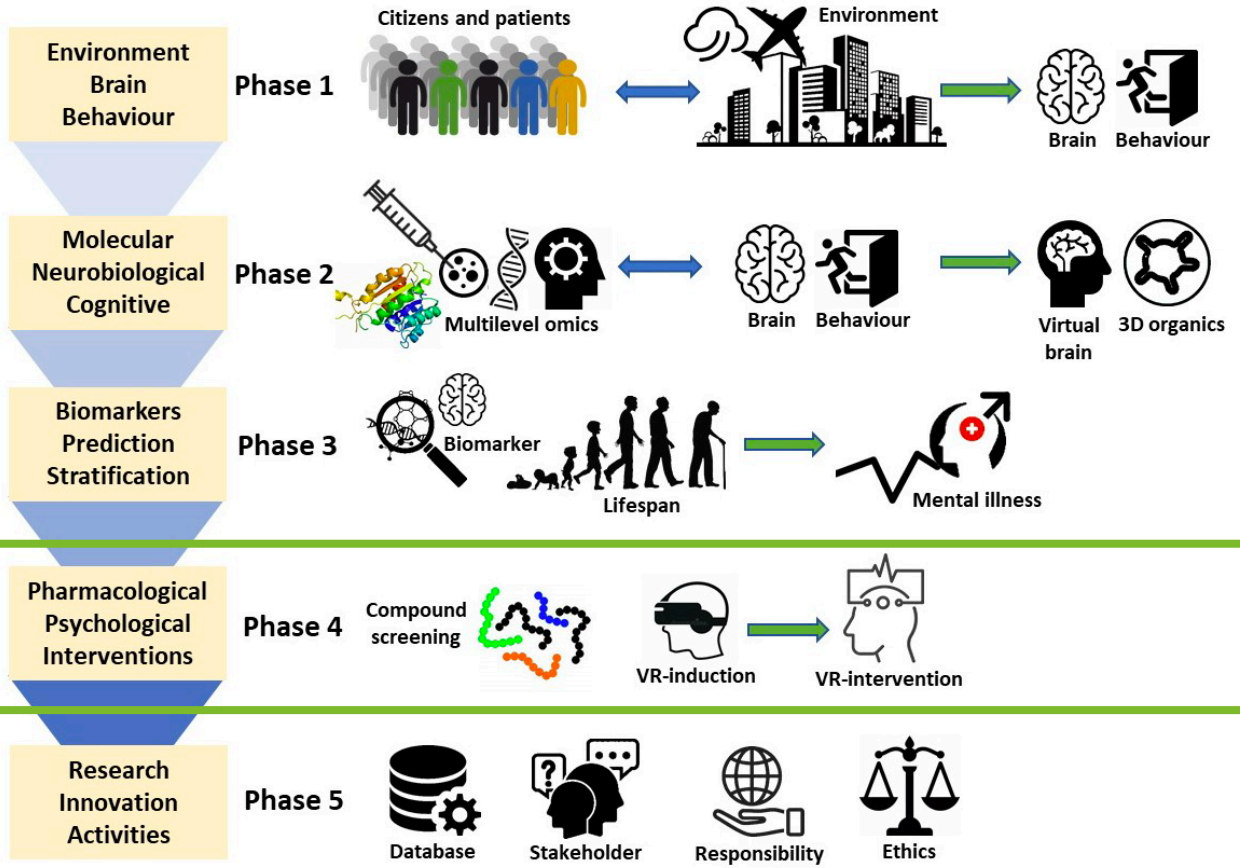

# Development of pharmacological, cognitive and educational interventions

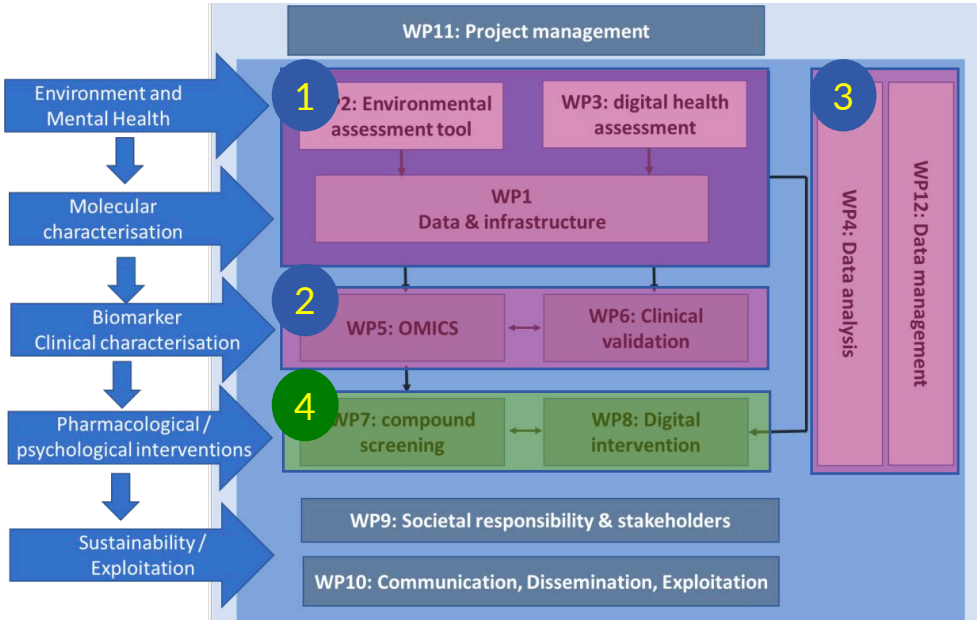
## environMENTAL seminar 4

# environMENTAL
## Reducing the impact of major environmental challenges on mental health



## Objective 4

Development of pharmacological, cognitive and educational interventions targeting molecular and neurobiological mechanisms of environmentally-sensitive symptoms of mental illness. (WP 7, 8)




Cellular disease modelling and exploration of pharmacological modulators of relevant neuronal phenotypes.

Digital health interventions based upon virtual reality (VR) programmes designed to develop adaptive coping strategies to the environmental risk profiles identified.

Dissemination and communication of research outcomes.

# Agenda:

Challenges and opportunities for drug discovery in mental disorders
Peter Sommer, PhD, Scientific Director, Ksilink, France

Implementation of a patient-derived disease model of Phelan McDermid Syndrome for the identification of SHANK3-specific chemical modifiers
Johannes Wilbertz, PhD, Head of Neuroscience, Ksilink, France

Using Virtual Reality for a Change
Mel Slater, PhD, Universitat de Barcelona, Facultat de Psicologia, Spain

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Session dedicated to EBRAINS


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patient-based drug discovery

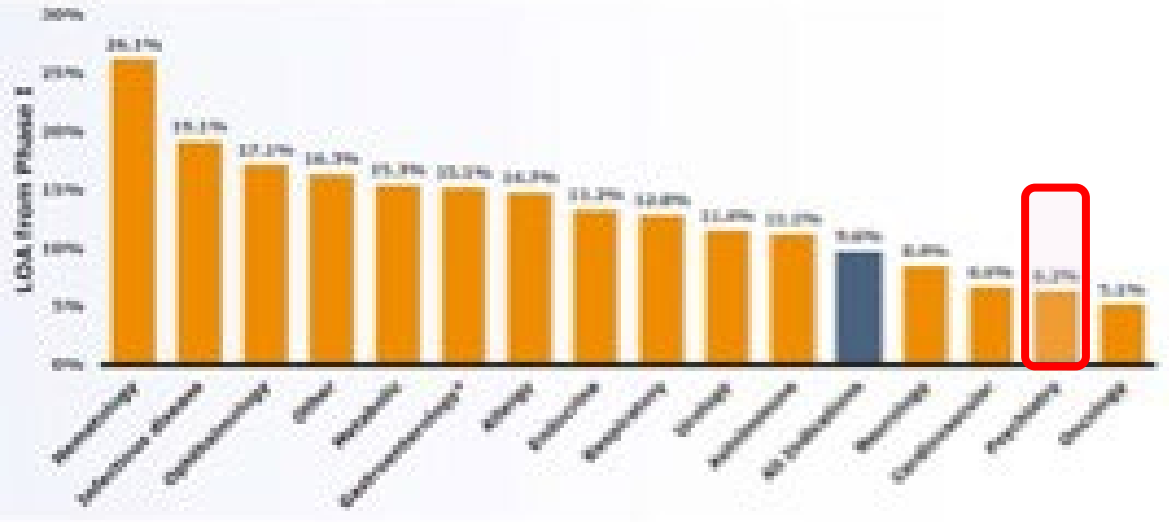
# Challenges and opportunities for drug discovery in mental disorders

Peter SOMMER, PhD
June 24th, 2022

# Drug Discovery & Development Challenges

The probability of success in clinical drug development for Psychiatric Diseases is comparably low to other disease areas

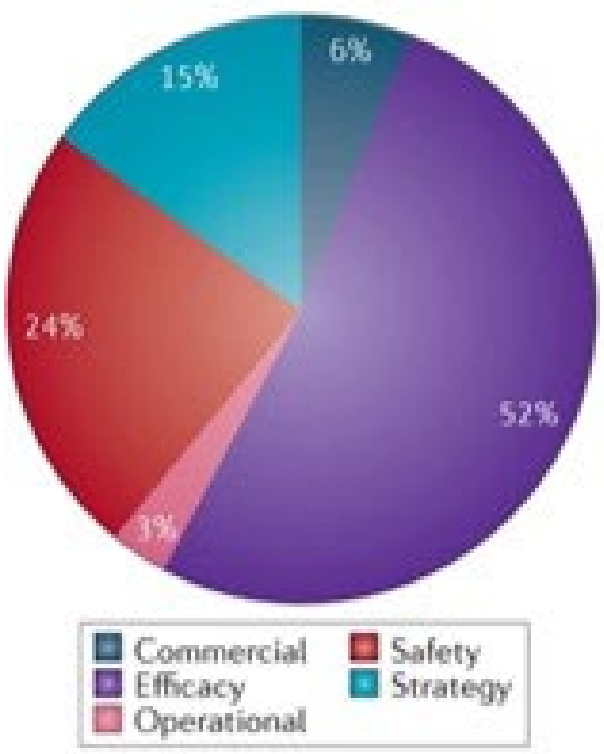
Likelihood of approval from Phase I by disease area



Thomas DW, Burns J, Audette J, Carroll A, Dow-Hygelund C, Hay M. Clinical Development Success Rates 2006–2015. Biotechnology Innovation Organization (BIO) Industry Analysis. (2016).

Increasing R&D costs alongside stagnating drug approval numbers and high failure rates in clinical development during the past decades coined the general notion of a “productivity crisis” in the pharmaceutical industry

# Drug Discovery & Development Challenges




Harrison, R. Phase II and phase III failures: 2013–2015. *Nat Rev Drug Discov* **15**, 817–818 (2016). https://doi.org/10.1038/nrd.2016.184

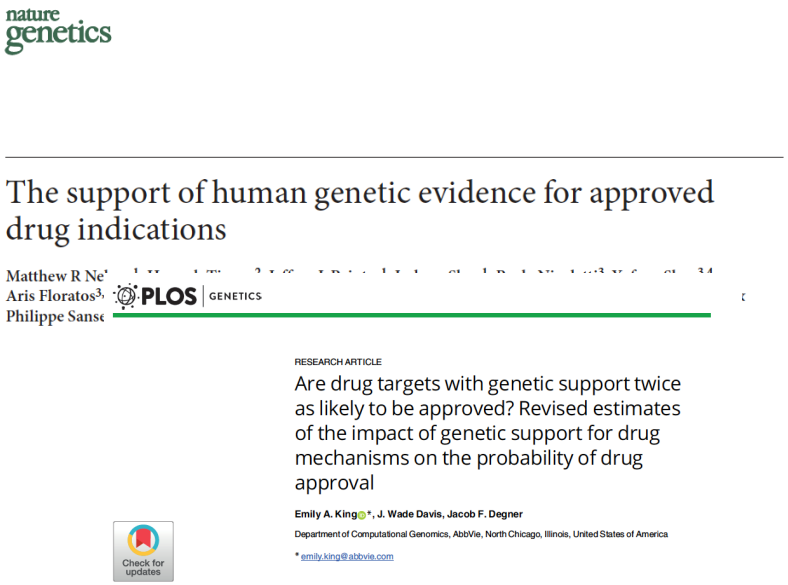
Major reasons attributed to the 90% clinical failures of drug development are:

- lack of clinical efficacy
- unmanageable toxicity
- poor drug-like properties

Improvement of clinical efficacy, pharmacology and safety requires authentic, predictive, translational models to support early drug discovery efforts before entering in expensive clinical trials.

Specific emphasis lies on the validation of the therapeutic strategy (target hypothesis & disease biology) necessitating extensive confirmation using genetic, genomic, and proteomic studies in cell lines, tissues, preclinical (animal) models, and human disease models.

# Drug Discovery & Development Opportunities


nature genetics

The support of human genetic evidence for approved drug indications

Matthew R Ne
Aris Floratos[3],
Philippe Sanse

PLOS | GENETICS

RESEARCH ARTICLE

Are drug targets with genetic support twice as likely to be approved? Revised estimates of the impact of genetic support for drug mechanisms on the probability of drug approval

Emily A. King*, J. Wade Davis, Jacob F. Degner

Department of Computational Genomics, AbbVie, North Chicago, Illinois, United States of America

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Check for updates

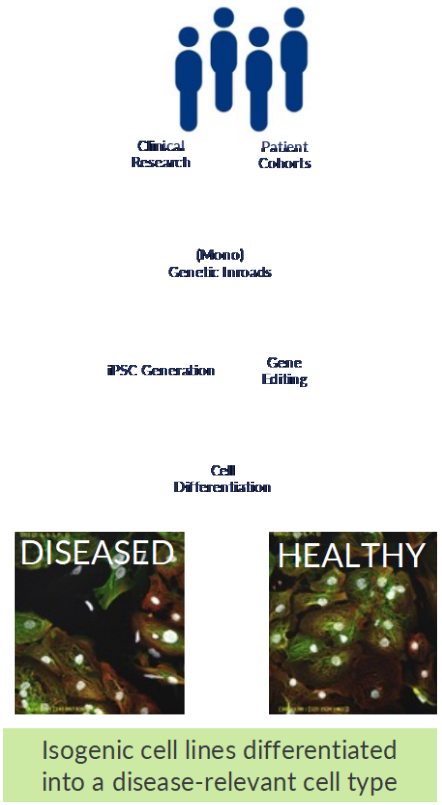
- Nelson et al., The support of human genetic evidence for approved drug indications. Nature Genetics. 2015;47(8):856. https://doi.org/10.1038/ng.3314
- King et al., Are drug targets with genetic support twice as likely to be approved? Revised estimates of the impact of genetic support for drug mechanisms on the probability of drug approval. PLoS Genet 2019;15(12):e1008489. https://doi.org/10.1371/journal.pgen.1008489

Impact of genetic support for drug mechanisms on the probability of drug approval in clinical phases

- Drugs with genetically supported targets were more likely to be successful in Phases II and III
- When causal genes are clear (Mendelian traits and GWAS associations linked to coding variants), **the use of human genetic evidence increases approval by greater than two-fold**.

# Patient-based disease modelling:

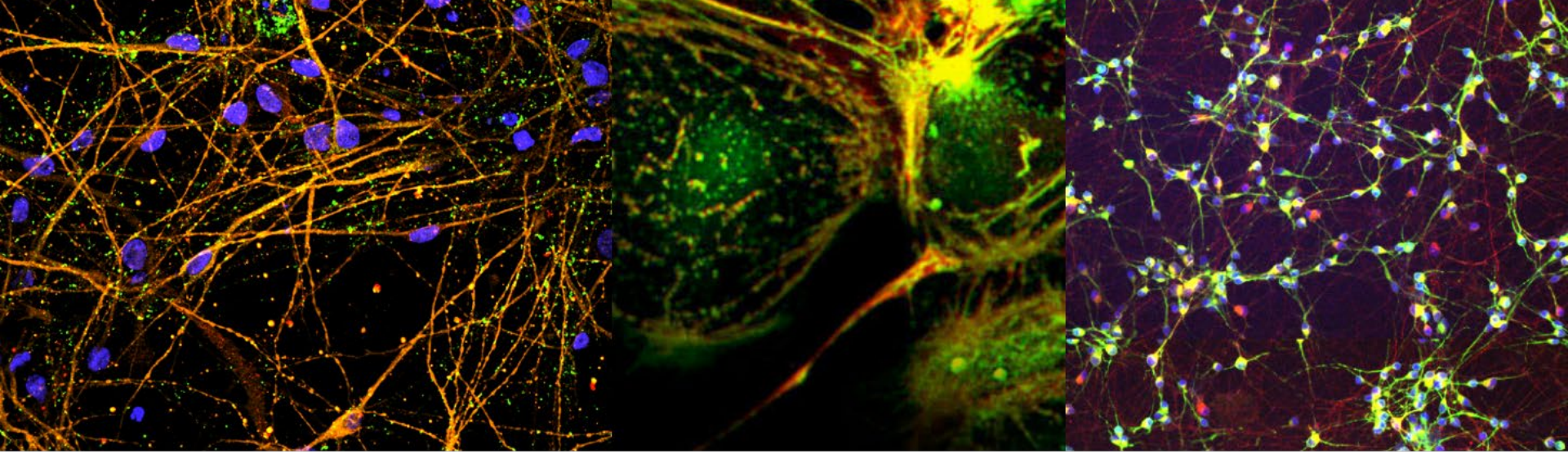
## An opportunity to overcome the productivity crisis within the pharmaceutical industry




Isogenic cell lines differentiated into a disease-relevant cell type

## Enabling technologies

- Large-scale sequencing and GWAS studies
- Increasing amount of multi-omics data sets including single cell and spatial resolution on the tissue level
- Reprogramming of somatic cells into self-renewable, pluripotent stem cells (iPSC
- Differentiation protocols (authentic cell types)
- Gene editing - ability to generate isogenic models and exploit (mono)genetic disease triggers
- High-end automated microscopy
- AI supported image and data analysis



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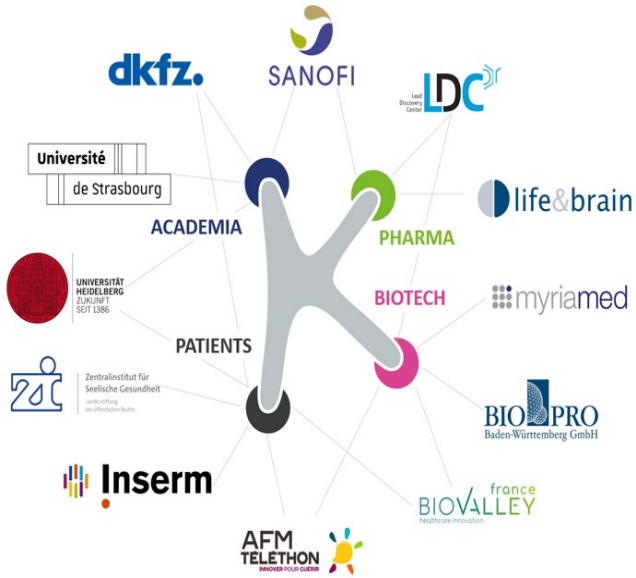
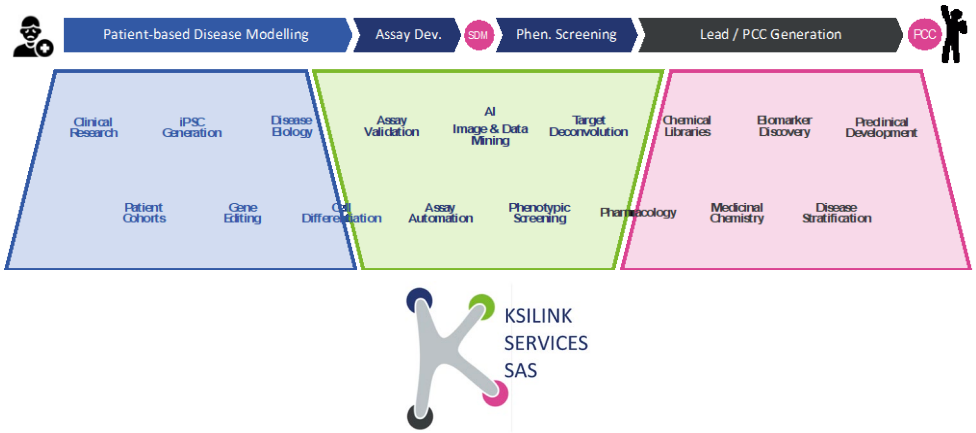
# Patient-based disease modelling:

## From genomic findings to cellular phenotypes

# KSILINK – the association



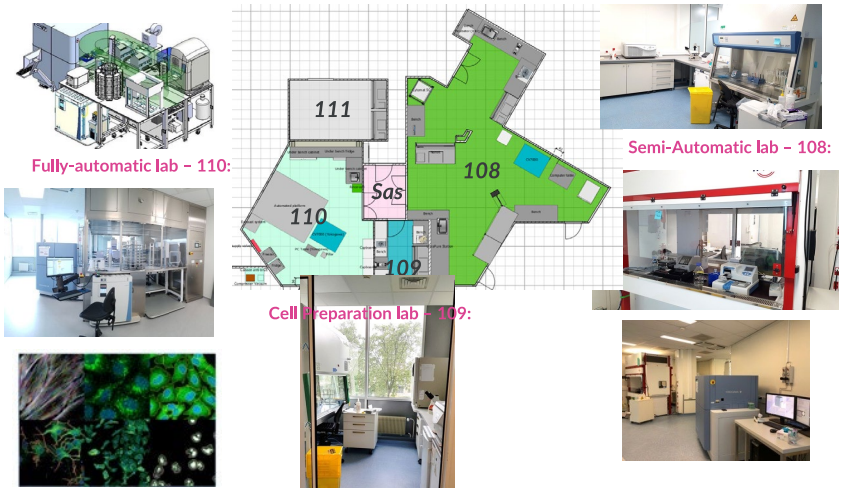



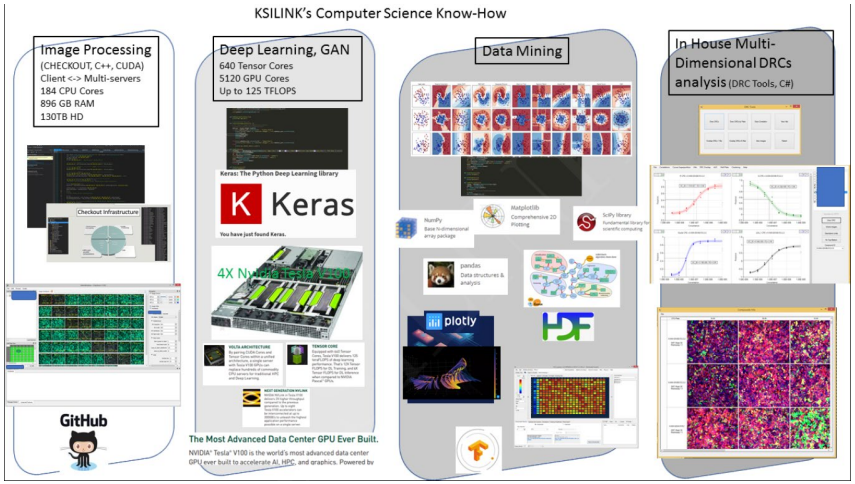

A French-German, public-private association dedicated to foster patient-based drug discovery

The association Ksilink owns Ksilink Services SAS, a dedicated service platform for high-content imaging and AI supported image & data analyses in Strasbourg, France.

Ksilink Services SAS is aligned with platforms from members of the association, bridging patient-based disease modeling capacities with drug discovery expertise for LEAD generation and preclinical development.

# KSILINK – platform technologies




## State-of-the-art high-content imaging facilities

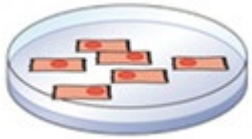
- High-throughput cytological discovery systems (Yokogawa CV7000) integrated in fully automated robotics platforms
- BSL2 environment and laboratory fume protection (i.e. PFA)
- Complementary assay technology including
  - Maestro Pro multi well microelectrode array (MEA) and impedance system
  - plate readers

## Cutting edge image and data analysis infrastructure

- Image Processing: proprietary C++ software termed CHECKOUT
- AI, Deep/Machine Learning, GPU computing: 280 cores, 8 GPUs V100 /RTX6000 (4,4) , 1TB of RAM, 1PTB Storage
- Integrated external (i.e. Cell Profiler) and in-house data analysis packages (i.e. HCS Analyzer)
- Specific in-house drug discovery and screening analysis tools, i.e. for multidimensional dose response curve analyses

# KSILINK – from Images to Data

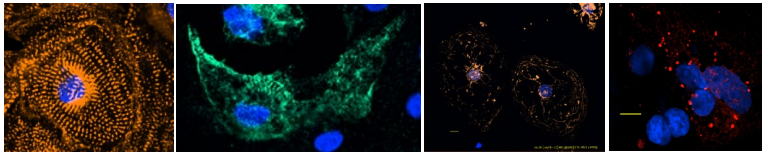
## Basic principles of the analytical workflow



Human
iPSCs-derived cells

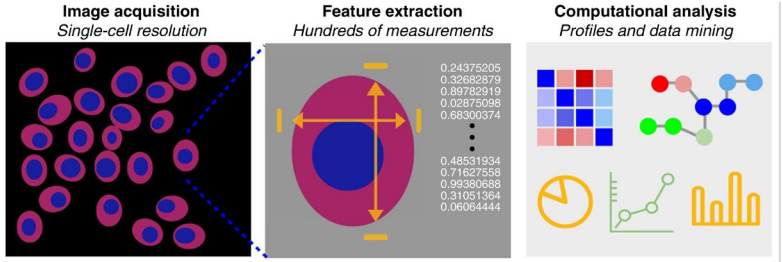

Automated Confocal
Microscopy

Immunohistochemistry



A-Actinin Nuclei | Desmin Nuclei | CoxIV Nuclei | p62 Nuclei

Multi-parametric image analysis & data mining




## High-content Imaging

- Miniaturized cell culture (2D, 2D+time, 3D, 3D+time)
- Labelling of cellular components of interest (fluorescence dyes, antibodies, fluorescence proteins, …)
- Image acquisition using automated confocal microscopy (4 fluorescence channels, bright-field)
- Image analysis: extraction of meaningful information from images by means of digital image processing techniques.
- Computational data analysis and data mining



Caicedo, J. C., Singh, S. & Carpenter, A. E. Applications in image-based profiling of perturbations. Curr. Opin. Biotechnol. 39, 134–142 (2016).

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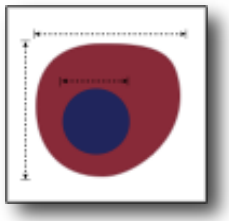
# KSILINK – from Images to Data

## Increasing the content of High-Content Imaging

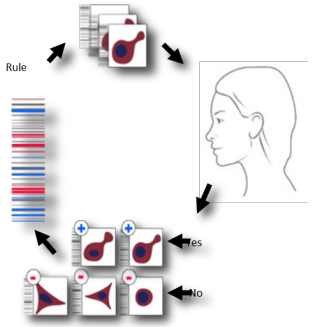
From classical segmentation-based quantitative morphometry to Machine/Deep Learning-assisted data mining and profiling

**1 Simple morphometric visible phenotype**



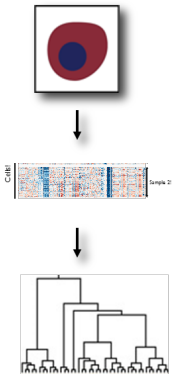
Write function

**2 Complex morphometric visible phenotype**



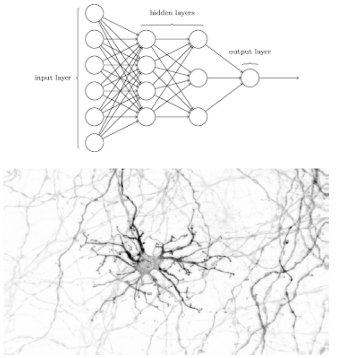

Train function

**3 Invisible/subtle phenotype on detectable objects**



Profiling

**4 Invisible/subtle phenotype on undetectable objects**




Deep learning

Interpretable ⟷ Powerful

# Early Drug Discovery process from conceptualization to clinical candidate


**Target Discovery**
*Concept for a new drug*

**Target Validation**
*Building confidence that the concept is valid*

**Screen Development**
*Set up a validated test panel to identify promising compounds*

**HIT Generation**
*Screen many compounds in the test panel*

**LEAD Development**
*Optimize identified HIT series*

**Candidate Identification**
*Seek compounds with drug potential (drug-like properties)*

**Candidate Drug Development**
*Safety evaluation/toxicology before dosing to man*

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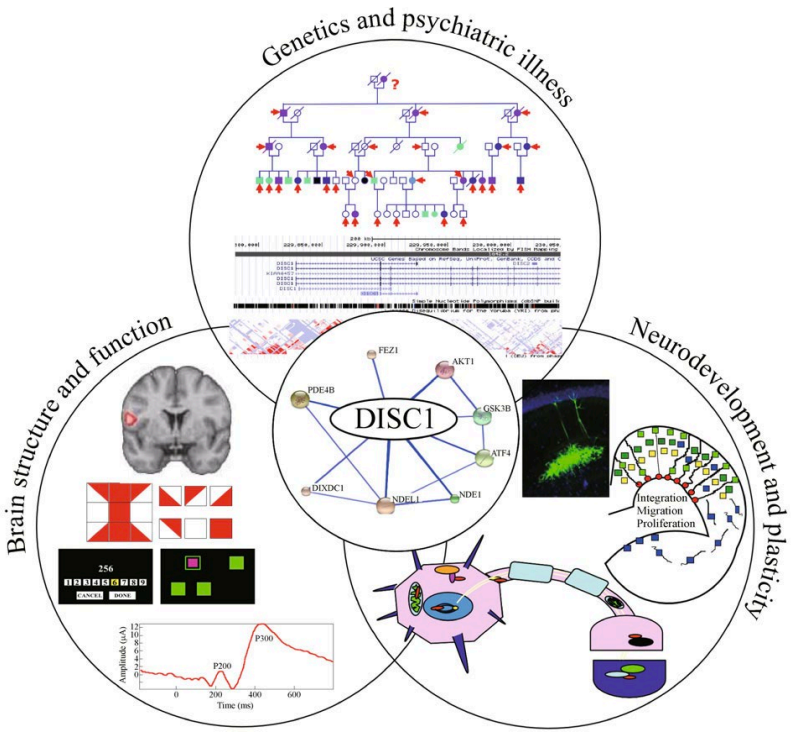
# Genetic inroads into psychiatric diseases

# Disrupted-in-Schizophrenia-1 (DISC1)

## A Key Susceptibility Factor for Major Mental Illnesses

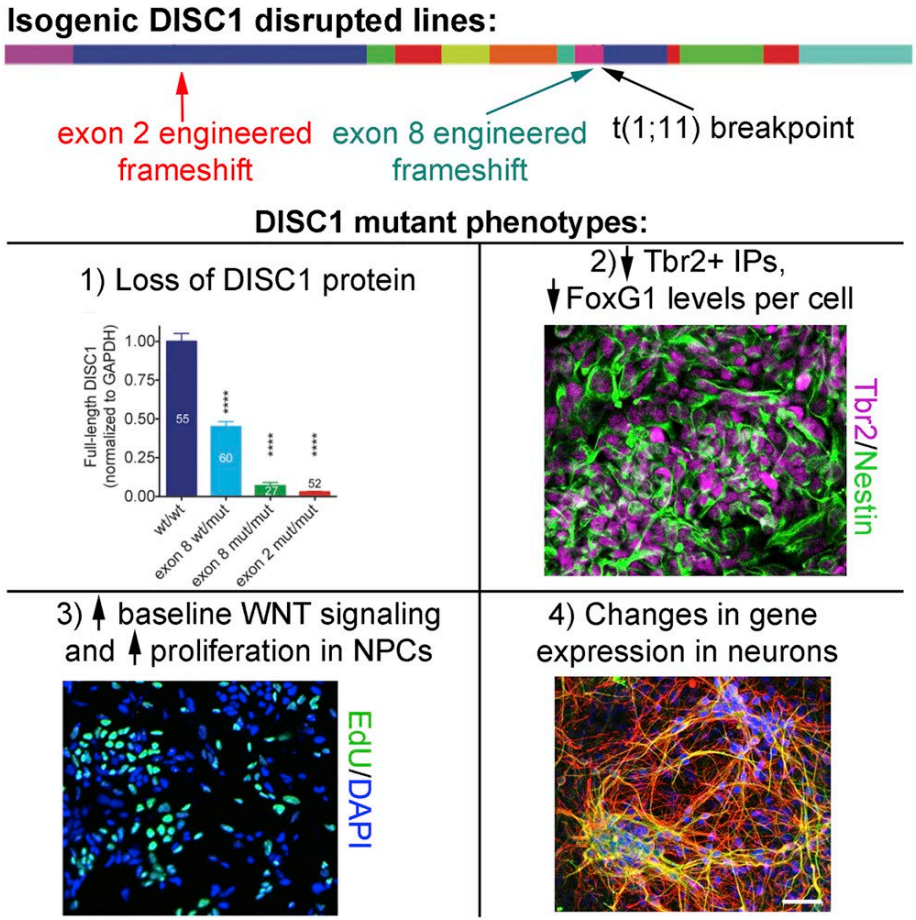



Several studies have shown that unregulated expression or altered protein structure of DISC1 may predispose individuals to the development of **schizophrenia, clinical depression, bipolar disorder, and other psychiatric conditions**.

The cellular functions that are disrupted by permutations in DISC1, which lead to the development of these disorders, have yet to be clearly defined and are the subject of current ongoing research.

A mutated *DISC1* gene constitutes an attractive candidate **to trigger** disease mechanisms and phenotypes relevant to major mental illnesses in an authentic cellular background

# *DISC1* as a trigger to model psychiatric diseases




Genetic and clinical association studies have identified disrupted in schizophrenia 1 (DISC1) as a candidate risk gene for major mental illness.

DISC1 is interrupted by a balanced chr(1;11) translocation in a Scottish family in which the translocation predisposes to psychiatric disorders.

DISC1 mutations result in reduced DISC1 protein expression and show subtle effects on certain presynaptic proteins.

Longitudinal analysis of neurite outgrowth revealed decreased neurite outgrowth in neurons with DISC1 mutation,


Transl Psychiatry. 2018 Apr 12;8(1):77
Transl Psychiatry. 2018 Nov 8;8(1):245.
Cell Rep. 2015 Sep 1;12(9):1414-29.

# *DISC1* as a trigger to model psychiatric diseases

Differentiation of iPSCs into forebrain-specific neural progenitors and cortical neurons.

Assessment and validation of DISC1-associated alterations

- **mutDISC1**
- **isoDISC1$^{wt}$**

Available via external resources

iPSC-derived post-mitotic neurons

**mutDISC1**

Targeted gene editing

**isoDISC1$^{wt}$**

**Multi-parametric profiling of mutDISC1 phenotype(s)**
- Diminished neuronal connectivity
- Decreased neurite number
- Diminuished neurite outgrowth
- Synaptic protein levels
- Glutamate receptor expression

**Pharmacological Rescue of mutDISC1 phenotype(s)**

**Genetic Rescue of mutDISC1 phenotype(s)**

**Complementary validation with gene expression profiles**

Assessment and validation with iPSC-derived neurons from patients and their 1$^{st}$ degree relatives

Patient-derived iPSCs

Generated at CIMH Mannheim

**Assessment and validation of common morphometric phenotypes**

**Complementary validation with gene expression profiles**

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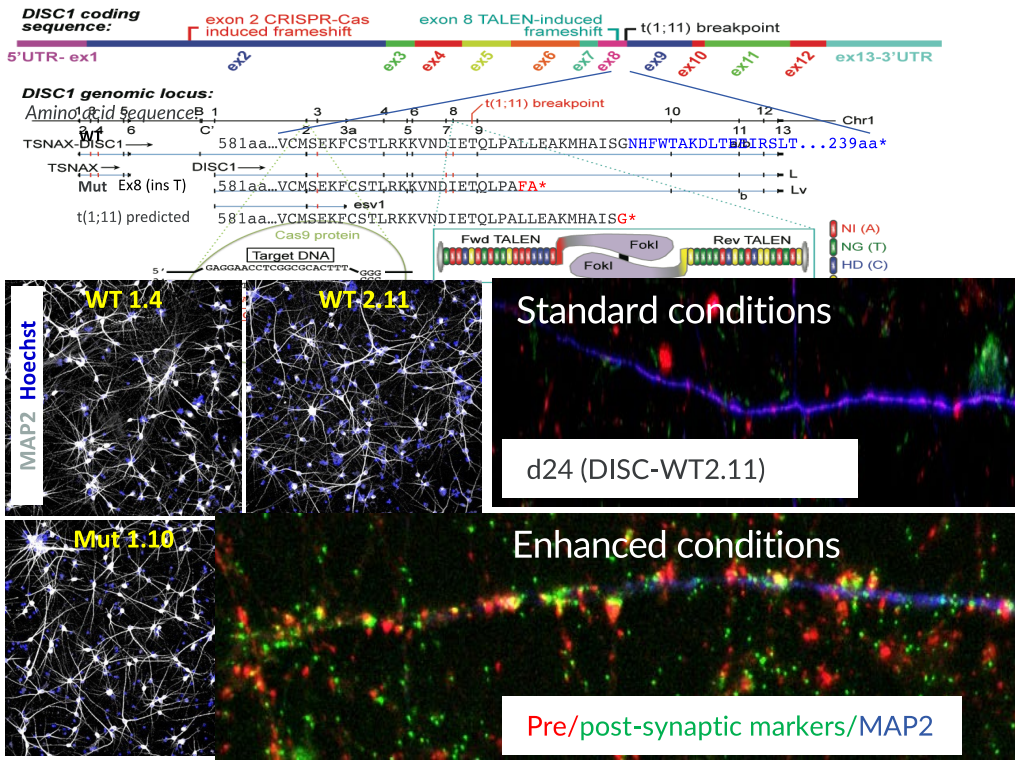
**Philipp Koch, Sandra Horschitz, Andreas Meyer-Lindenberg**

# *DISC1* as a trigger to model psychiatric diseases



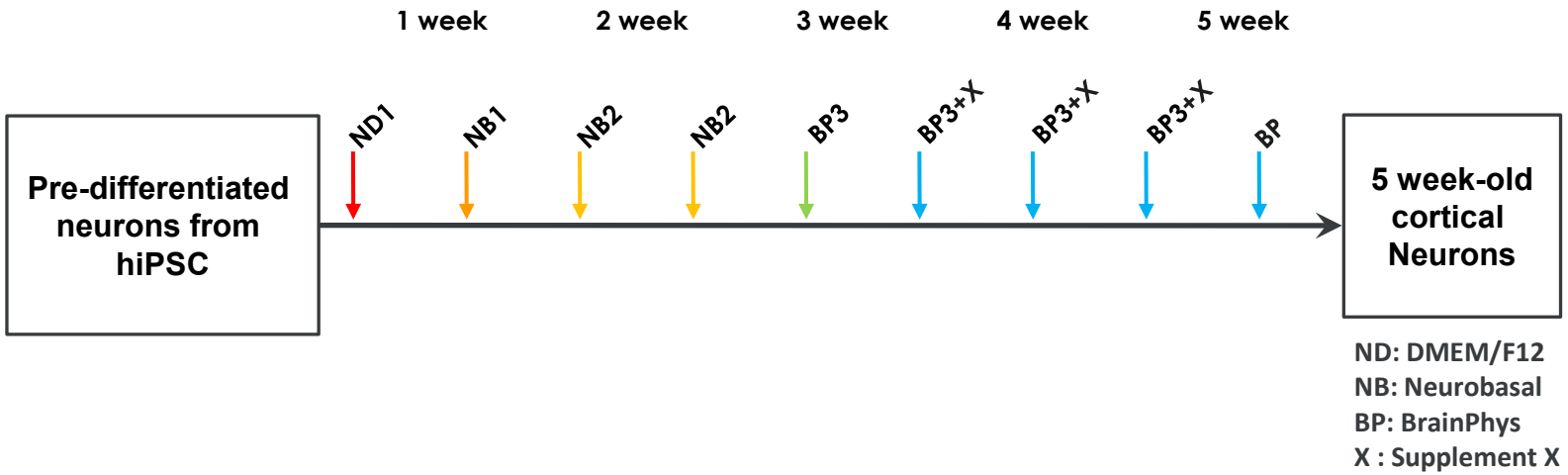

- Isogenic iPSC panel with an engineered, heterozygous t(1;11) translocation in DISC1
- Generation of glutamatergic neurons with cortical projection neuron identity (> 90% Tau+, < 6% GFAP+, > 80% Tbr1+and/or Ctip2+)
- Purities >90%
- Minimal variation between batches and cell lines
- Freezing procedure allowing high thawing efficiencies
- Protocols for fast and controlled maturation (expression of mature synaptic proteins in time window for screening)

# *DISC1*-triggered developmental synaptopathologies

Development of a culture protocol that can boost synapse formation of cortical neurons in a relatively short time periods (35 days)

Miniaturized format (384-well plates) and largely automatized workflow

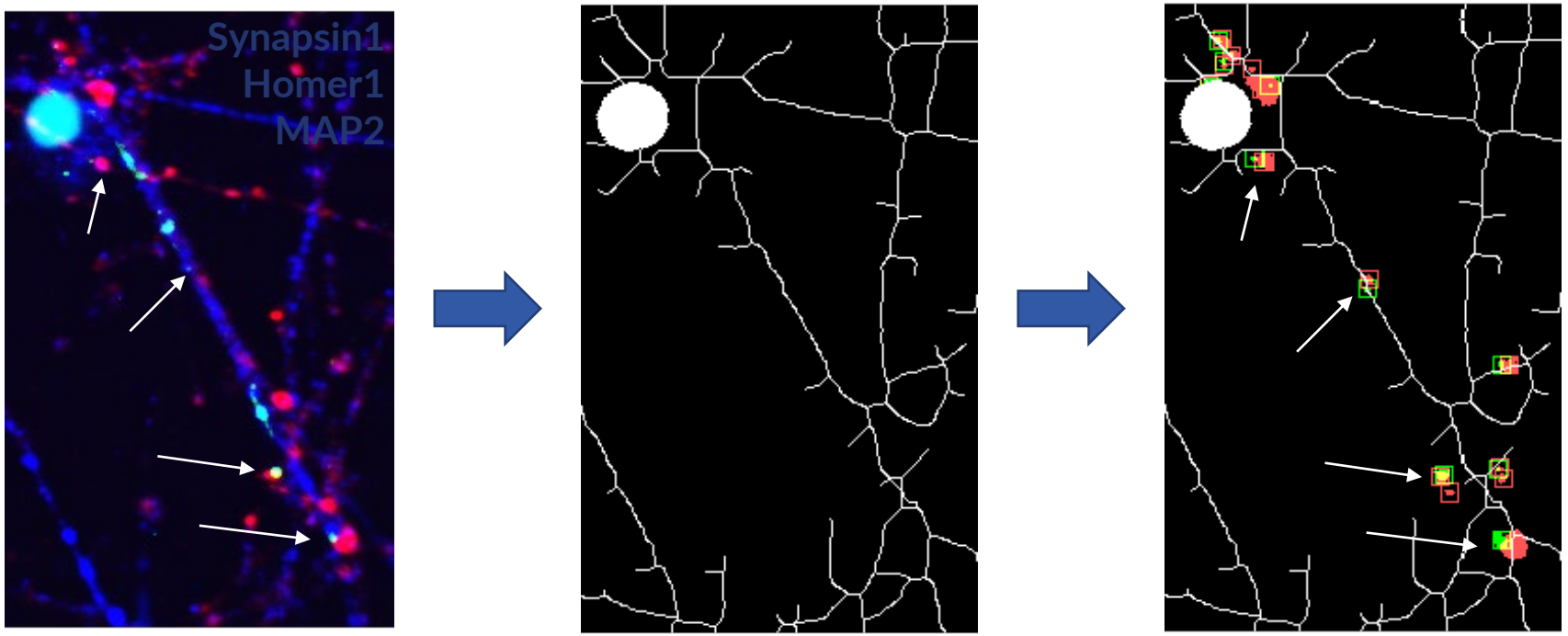
Determination of **neuronal complexity** and **synapse formation** in cortical neurons



# *DISC1*-triggered developmental synaptopathologies

## Detection of structural synapses

- Skeletonization of the MAP2 staining (dendrites)
- Drawing of a 10 px square box around the detection.
- Intersection between Synapsin1 box (presynaptic) and Homer1 box (postsynaptic) score as a synapse



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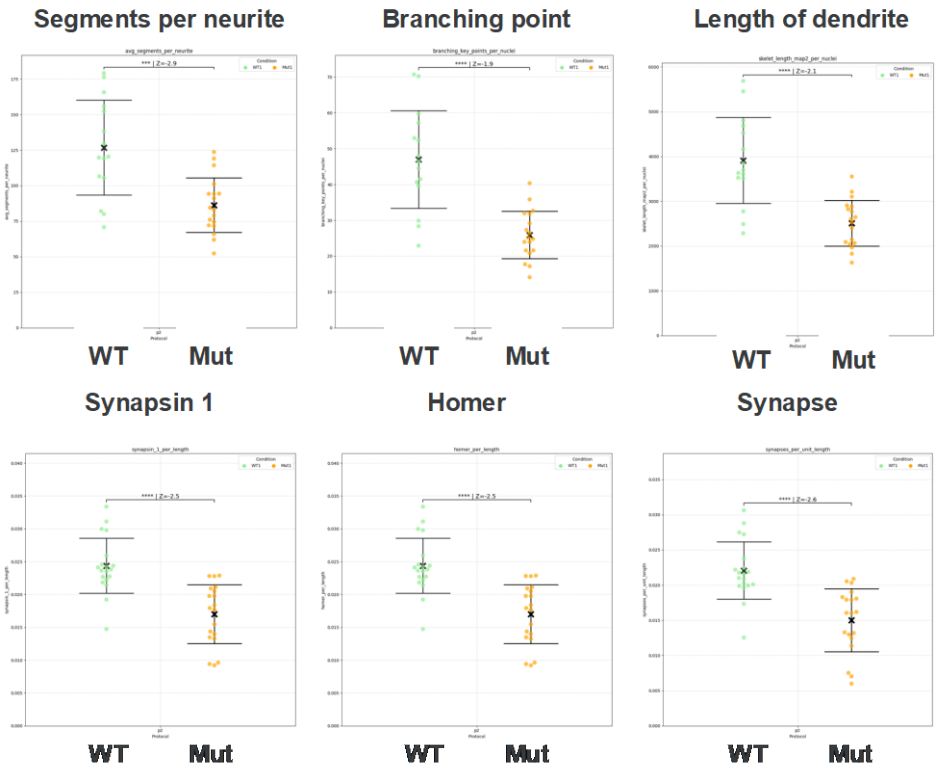
# *DISC1*-triggered developmental synaptopathologies

## Morphometric quantification of individual parameters




Synapse quantification is basically based on the quantification of pre- and postsynaptic compartments and their association.

Furthermore, in order to normalized synapse number, multiple morphometric features, such as structural components of the neuronal network (i.e. dendrite lengths) and cell numbers are taken into account

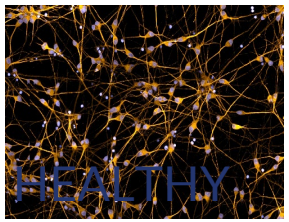
# *DISC1*-triggered developmental synaptopathologies

## Multiparametric analysis of morphometric quantification data

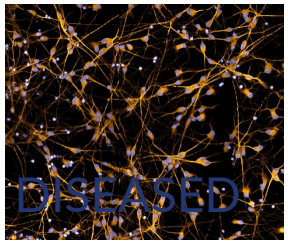
### LDA (Linear Discriminant Analysis)

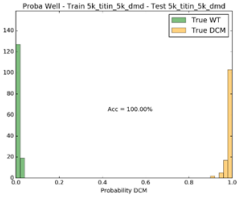
- finding a linear combination of features that separates “Healthy” from “Diseased”

WT



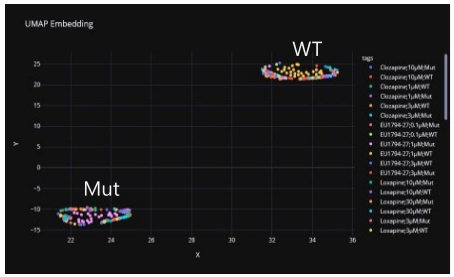

Mut DISC1




### UMAP (Uniform Manifold Approximation and Projection) for Dimension Reduction

- The method deforms the space locally preserving the global structure from N dimensions to 2 dimensions.
- Due to its properties the method is powerful to separate the data in different classes as WT vs mut. BUT doesn’t preserve the initial distances.



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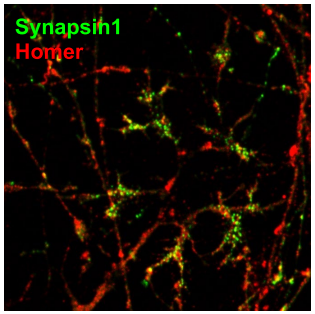
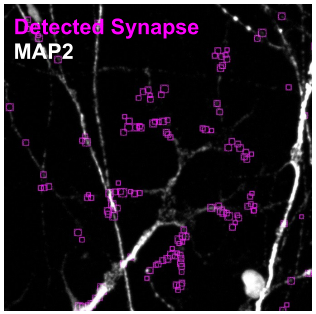
# *DISC1*-triggered developmental synaptopathologies

## Multiparametric analysis of morphometric quantification data

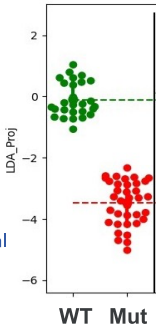



**LDA analysis based on:**

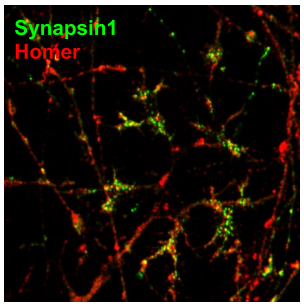
1) 6 features about synapses formation

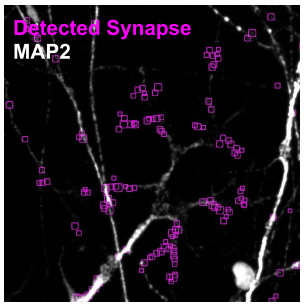
2) 4 features about neuronal network complexity



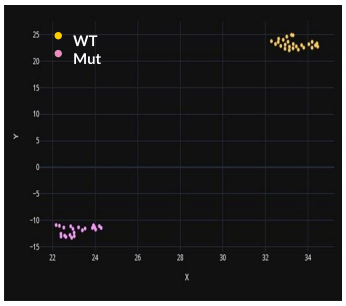

1) 'synapsin_1_per_nuclei', 'homer_per_nuclei', 'synapsin_1_per_length', 'synapses_per_unit_length', 'synapses_per_nuclei', 'homer_per_length'

2) 'skelet_length_map2_per_nuclei', 'branching_key_points_per_nuclei','end_points_per_nuclei', 'start_points_per_nuclei',




**UMAP embedding**




Both, LDA analysis and UMAP embedding separate the two classes (WT & Mut) a statistical power that can allow screening efforts.



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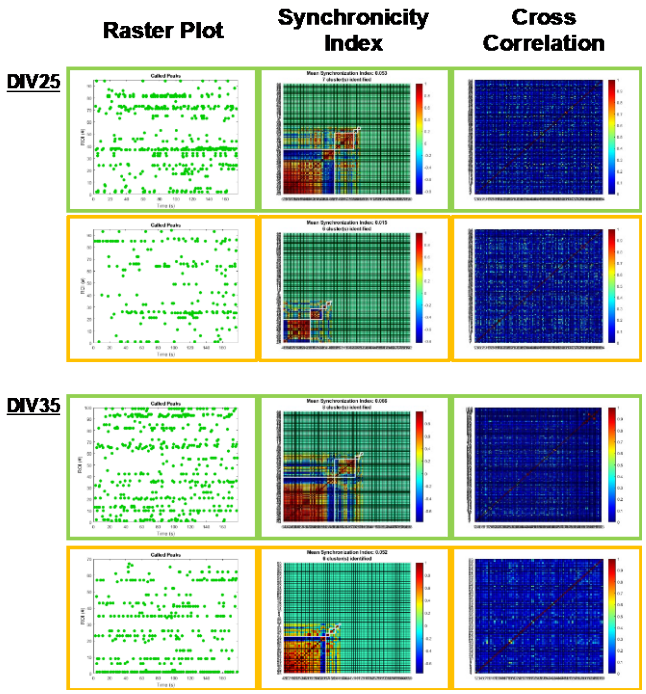
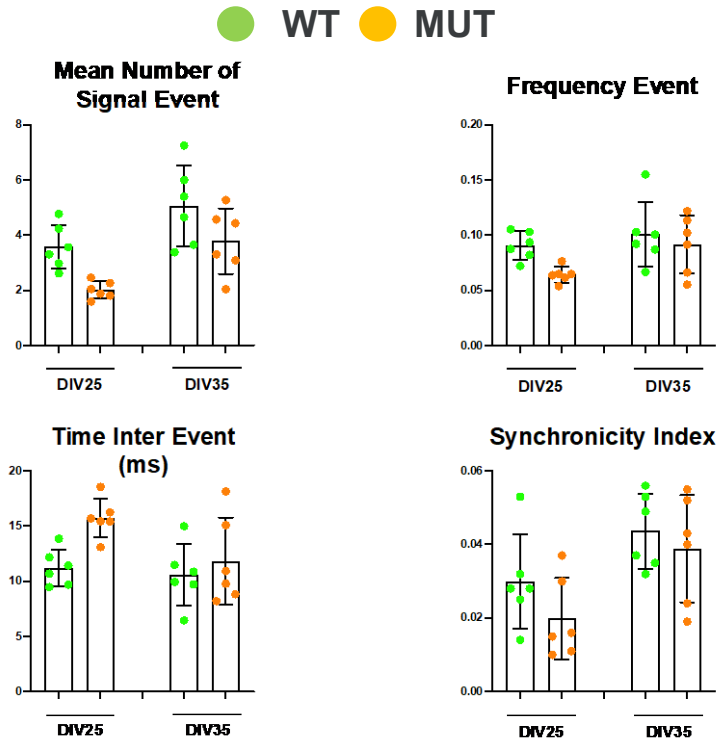
# *DISC1*-triggered developmental synaptopathologies

## Functional endpoints corroborate the observed synaptic phenotype




Analysed by PeakCaller (MatLab script)




Calcium imaging confirms reduced spontaneous activity in DISC1 mutated cells. At the assessed time points, cultures show low synchronicity and low network activity suggesting a developmental phenotype.



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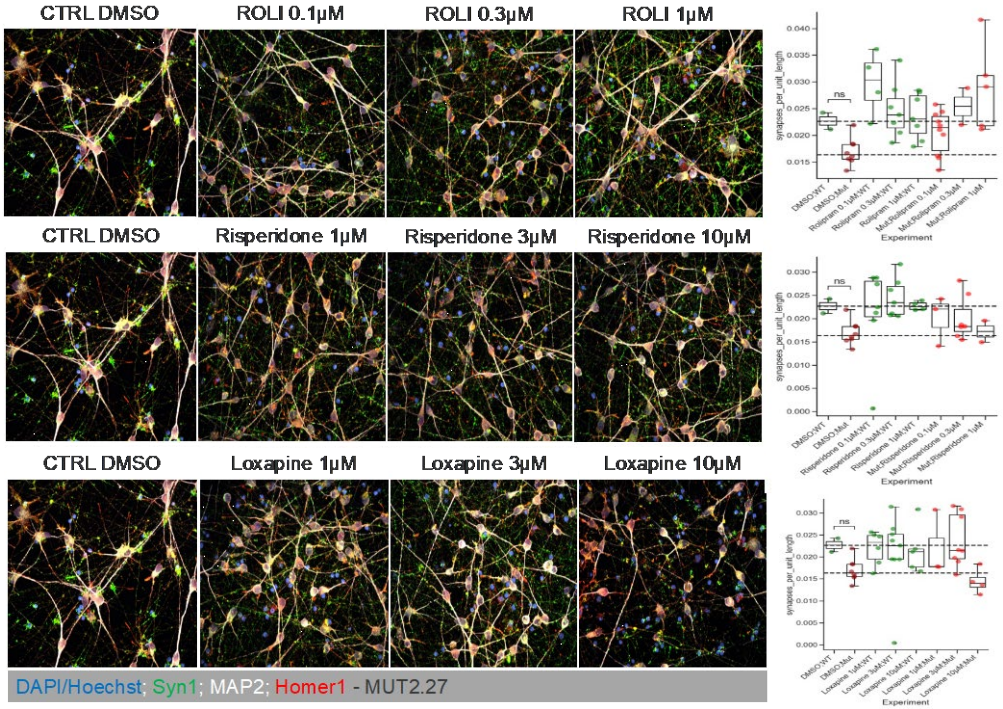
# *DISC1*-triggered developmental synaptopathologies

## Pharmacological modulation of structural synaptic endpoints







Pharmacological treatment can modulate structural, synaptic phenotypes in an isogenic disease model triggered by DISC1 mutation.

Cortical neurons were treated for 7 days with Rolipram (PDE4 inhibitor) or the (atypical) antipsychotic drugs Risperidone and Loxapine (D2/D3 and 5-HT2A antagonists).



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# Summary and perspectives

*DISC1*-triggered developmental synaptopathologies can be recapitulated in an iPSC-derived cortical neuron model

We confirm a causal link between a rare patient mutation in *DISC1* and synaptic deficits in cortical neurons differentiated from isogenic iPSCs.

Pharmacological inhibition of the PDE4 signalling pathway as well as antagonists of dopamine D2 receptors and at serotonin (5-HT) receptors rescue synaptic abnormalities.

Corroboration of the observed structural phenotypes by functional endpoints (Ca-imaging, electrophysiology, etc) are in progress.

In depth characterization and validation of the authenticity, predictivity and translatability of the *DISC1*-triggered cellular disease model including a comparative assessment of common morphometric and functional phenotypes using iPSC-derived neurons and post-mortem tissue from idiopathic patients.

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# THANK YOU!

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