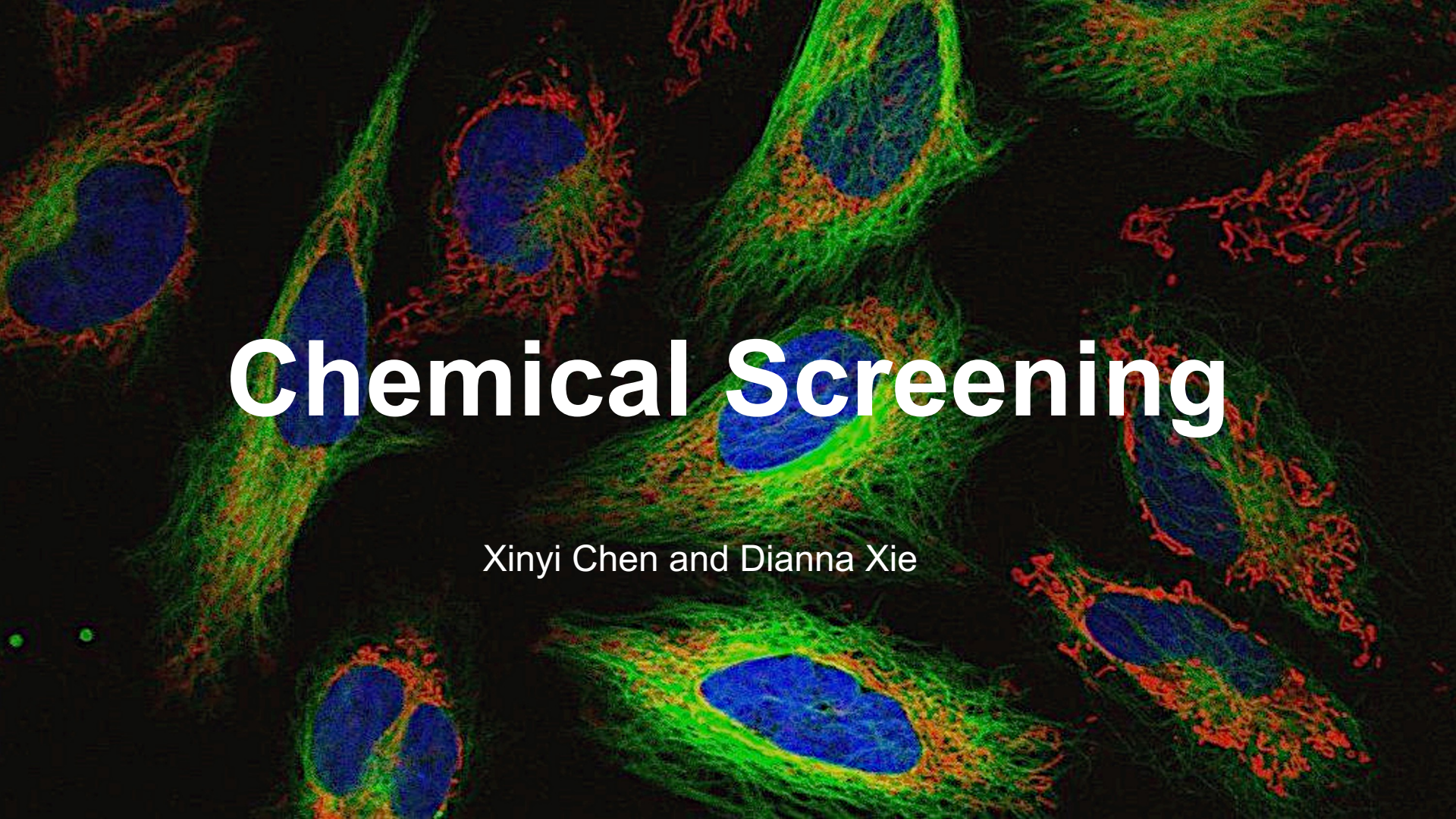
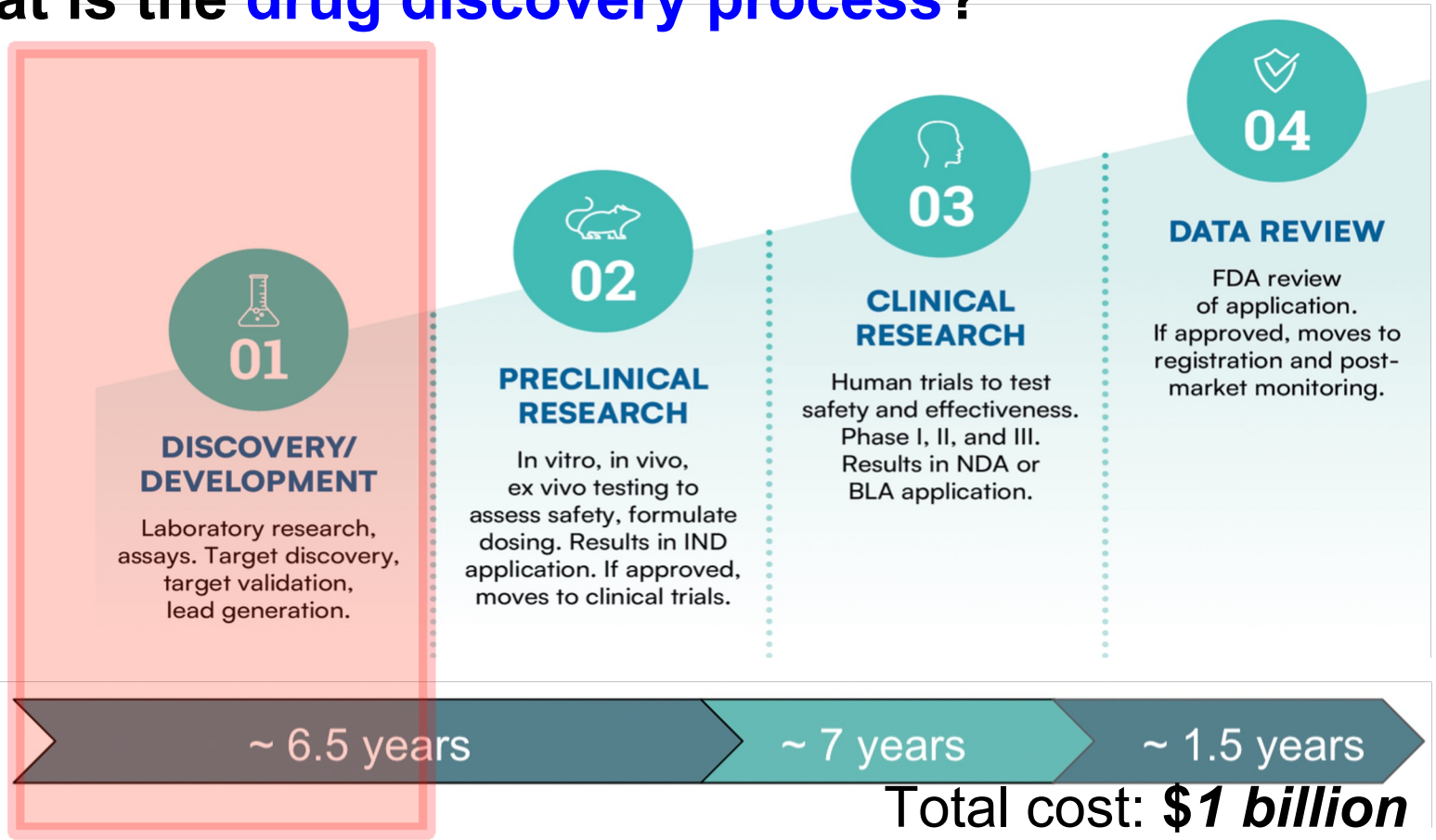


Chemical Screening

Xinyi Chen and Dianna Xie

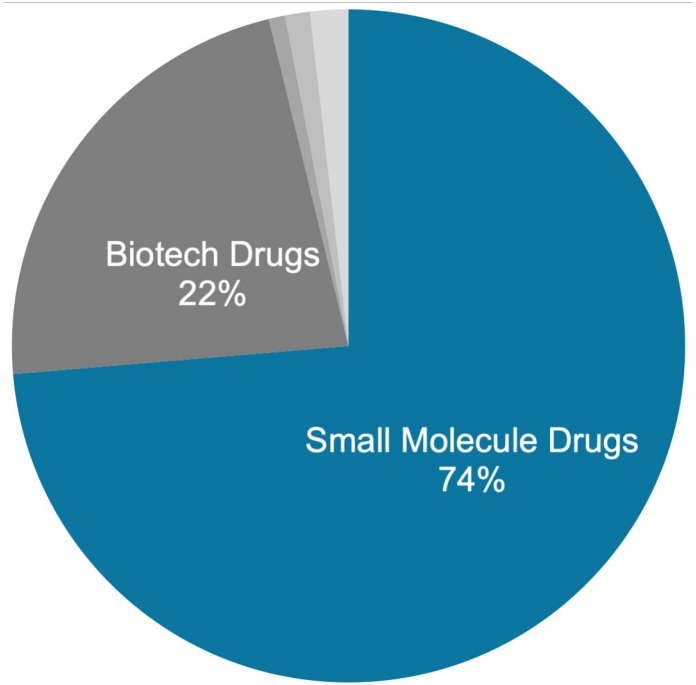
What is the drug discovery process?



Total cost: ***$1 billion***

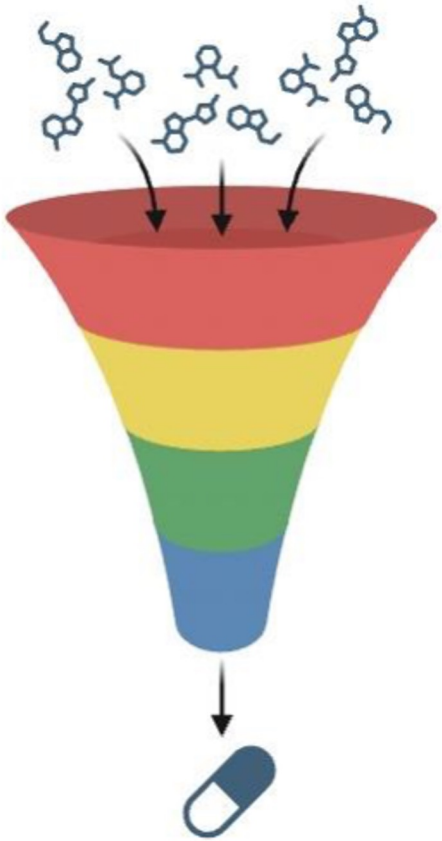
Development: **> $4 *million***

What is a small molecule drug?



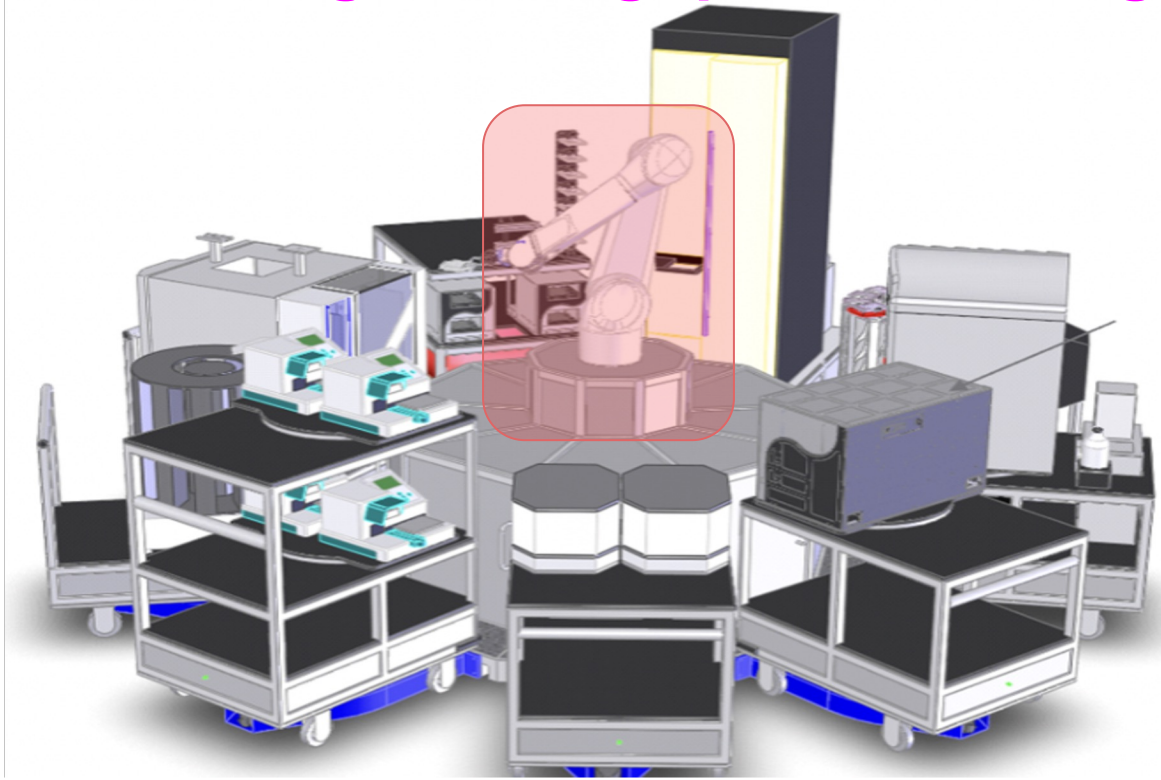
Small molecules are organic compounds with low molecular weight(< 1 KDa)

What is chemical screening?



Chemical screening refers to the process of testing a series of chemical compounds to identify those that have the desired biological activity

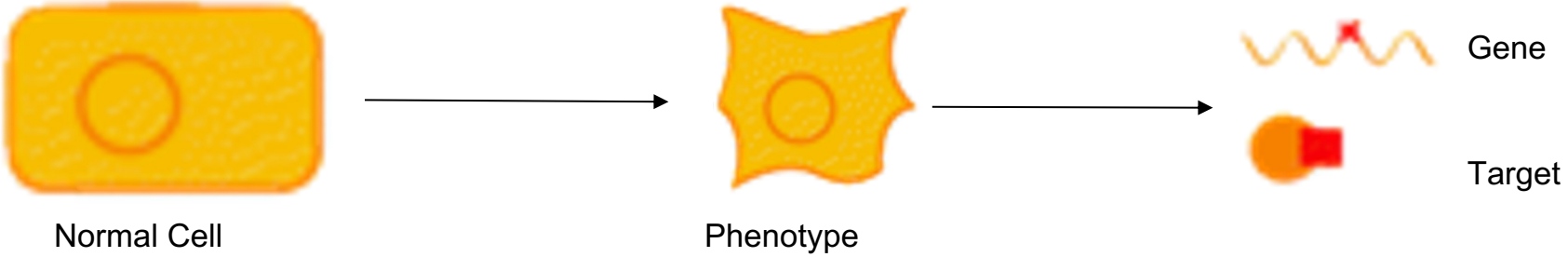
What is High-throughput Screening?



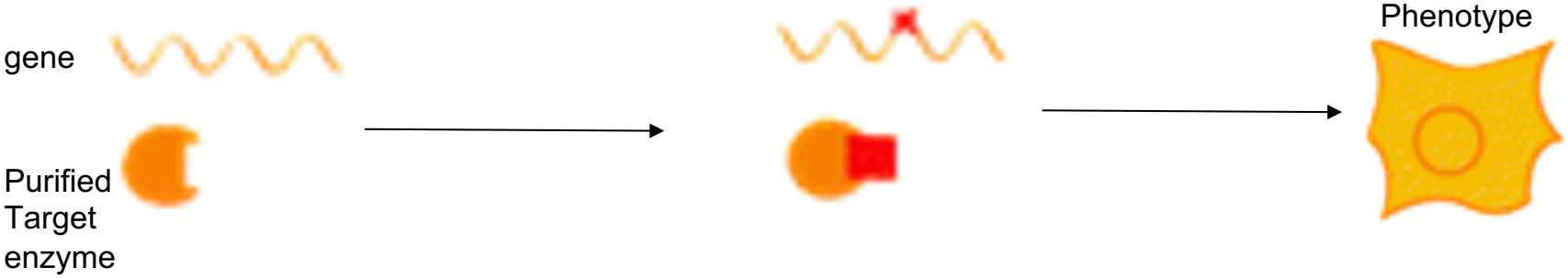
HTS is a high-tech way to accelerate the drug discovery process, allowing quick and efficient screening of large compound libraries at a rate of a few thousand compounds per day or per week.

Two pathways of chemical screening

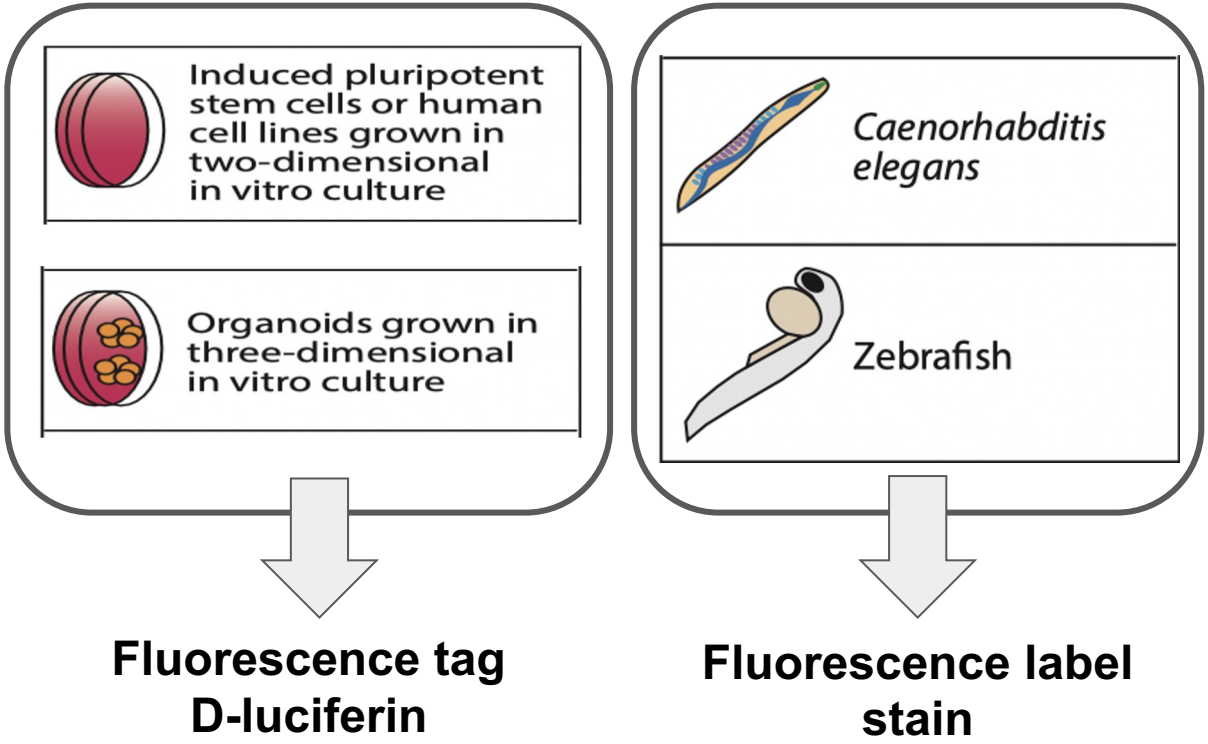
a. Forward Chemical Screening = Phenotypic Screening



b. Reverse Chemical Screening = Target-Based Screening

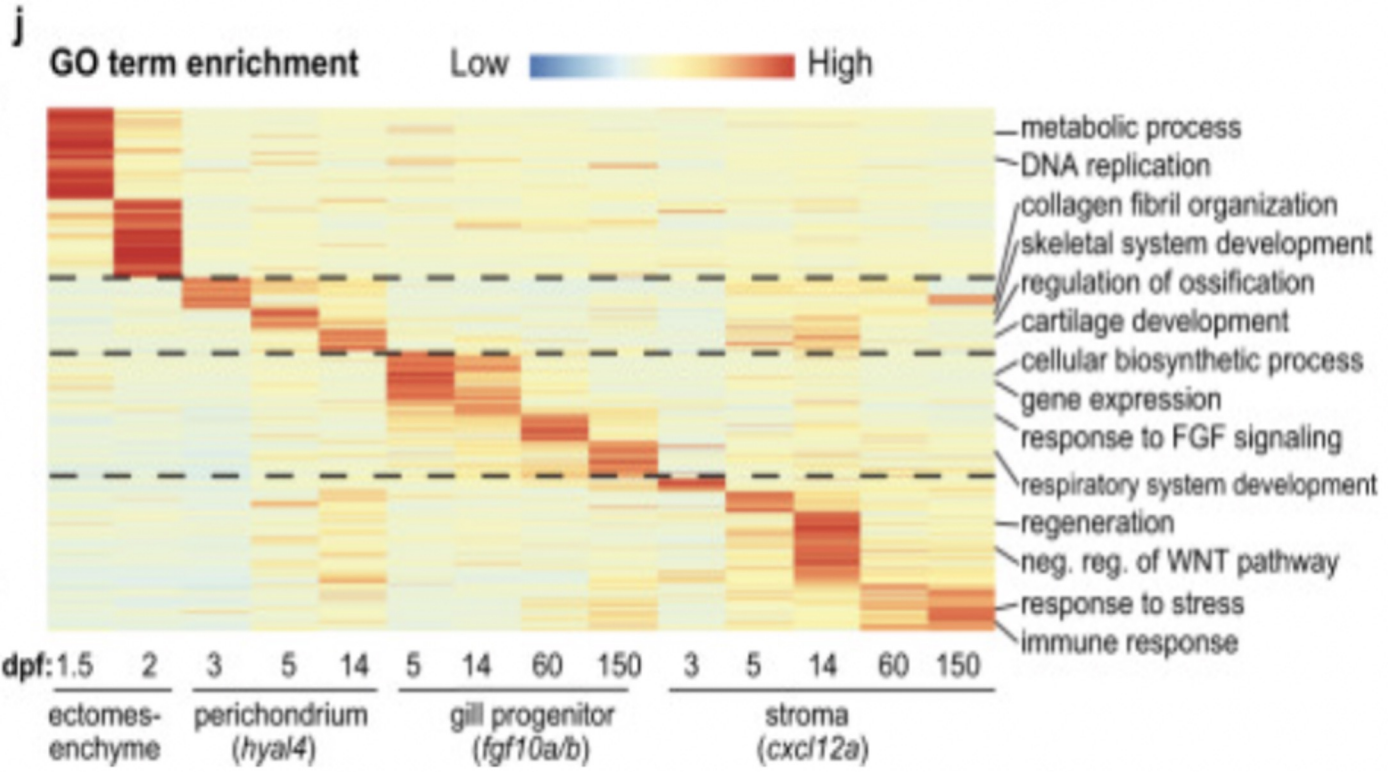


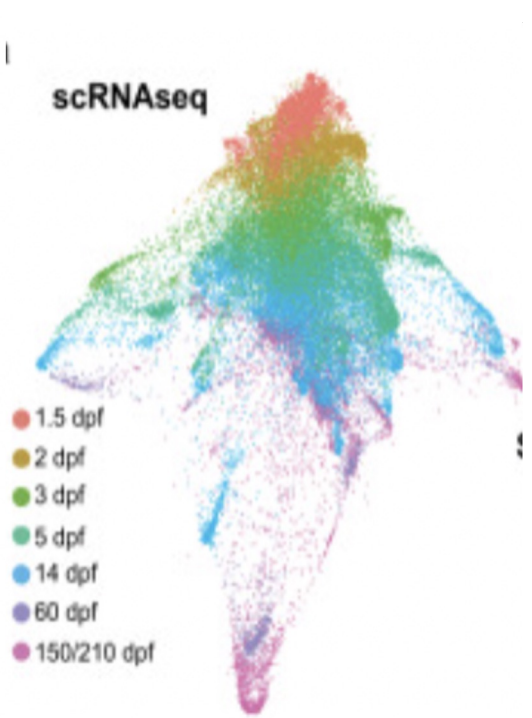
How do you prepare samples for forward chemical screening?



Dang et al., 2016
Fábián et al., 2022

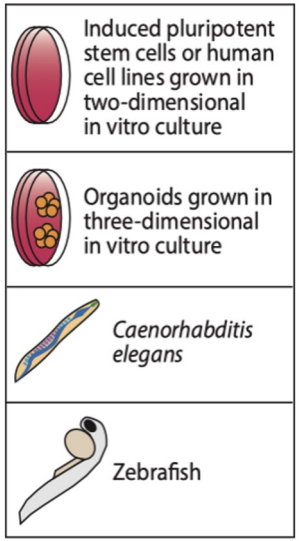
How do you prepare samples for forward chemical screening?

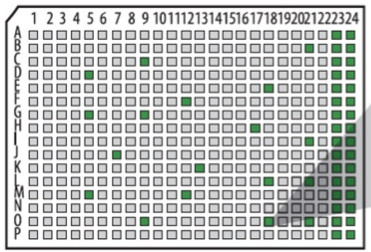


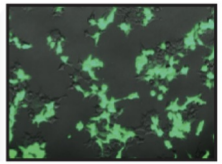


Dang et al., 2016
Fábián et al., 2022

What are the steps of forward chemical screening?







Target engagement?

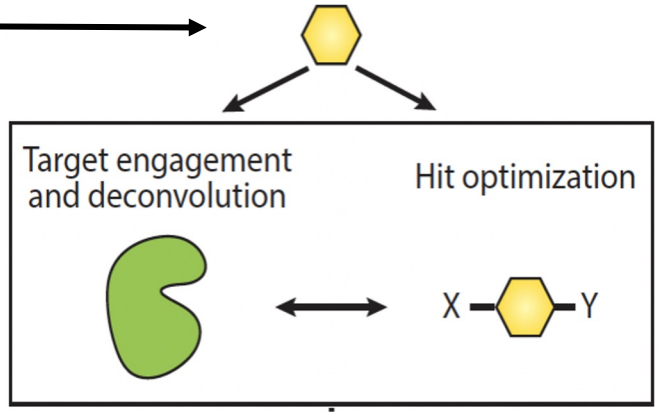
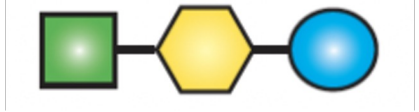


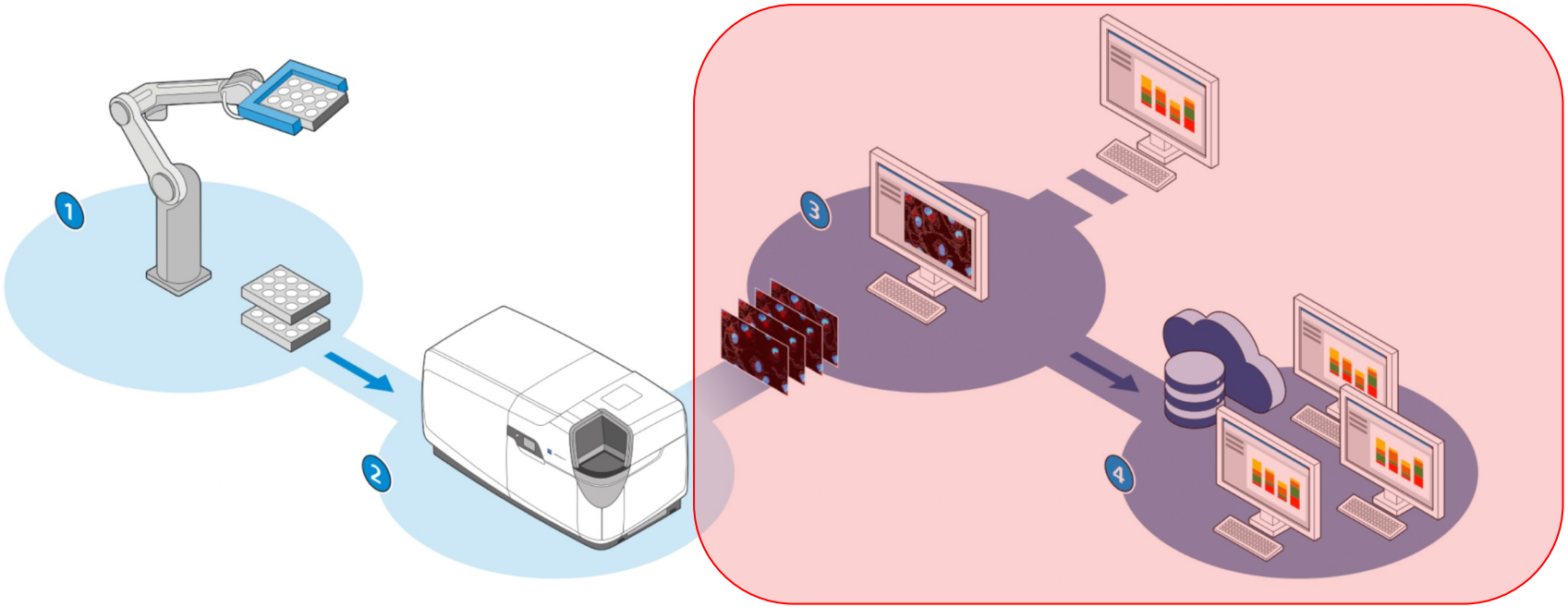
Plate Reader	Imaging
FINT, FRET,FP LUM	FACS Epi-FINT **HCA**

Hit to lead



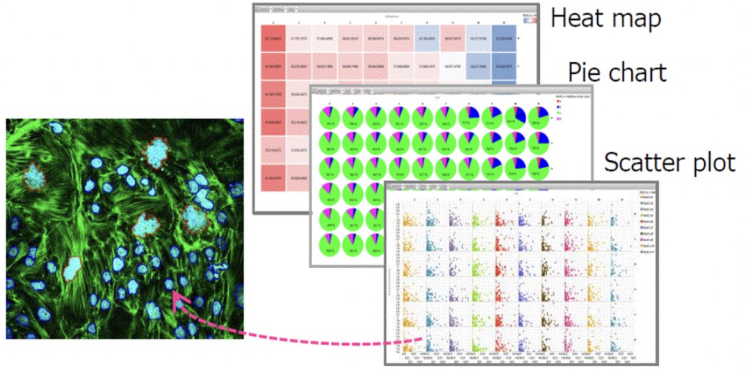
Dang et al., 2016

What is High-Content Analysis?

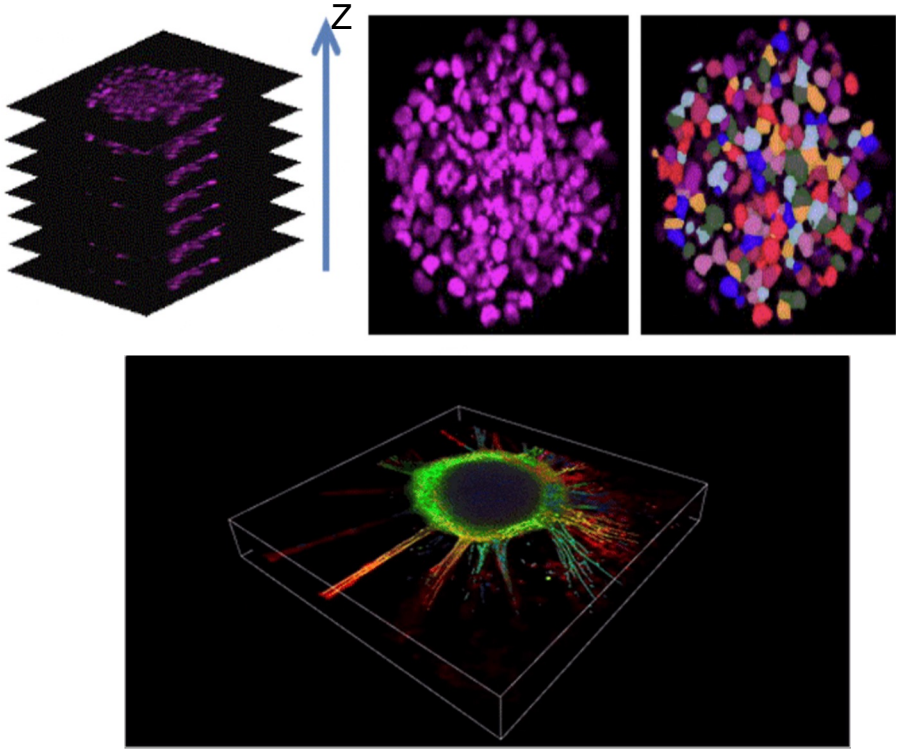


HCA combines *automated imaging* and *quantitative data* analysis in a high-throughput format to produces a large number of *individual* cell measurements.

What are the advantages of HCA?



Multiparametric records facilitate reanalysis



Multicellular structures from 3D spheroids, Organoids

What are Softwares for High-Content Analysis?

CellProfiler™ cell image analysis software → Cell counts, sizes, morphologies

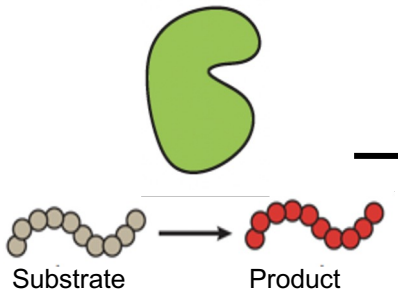
ADVANCED CELL CLASSIFIER → Classification of cell images

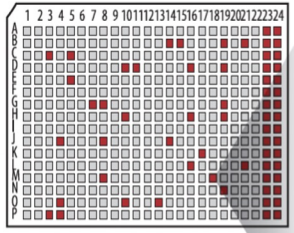
DEFINIENS Understanding Images → Digital pathology

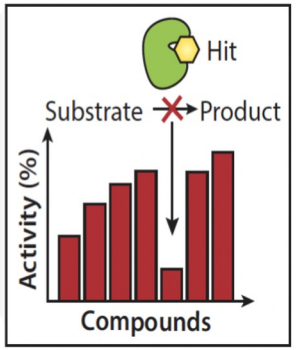
Core Life Analytics → No advanced knowledge data analysis techniques required

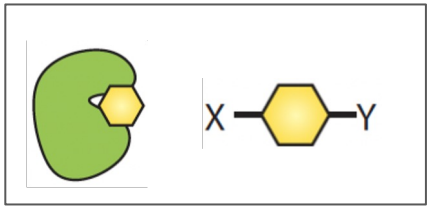
Phenonaut → Phenotypic analysis

What are the steps of reverse chemical screening?





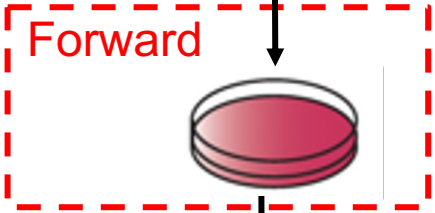




Biological context?

Plate Reader
ABS FINT, FRET,FP LUM

Biophysical
SPR MS NMR



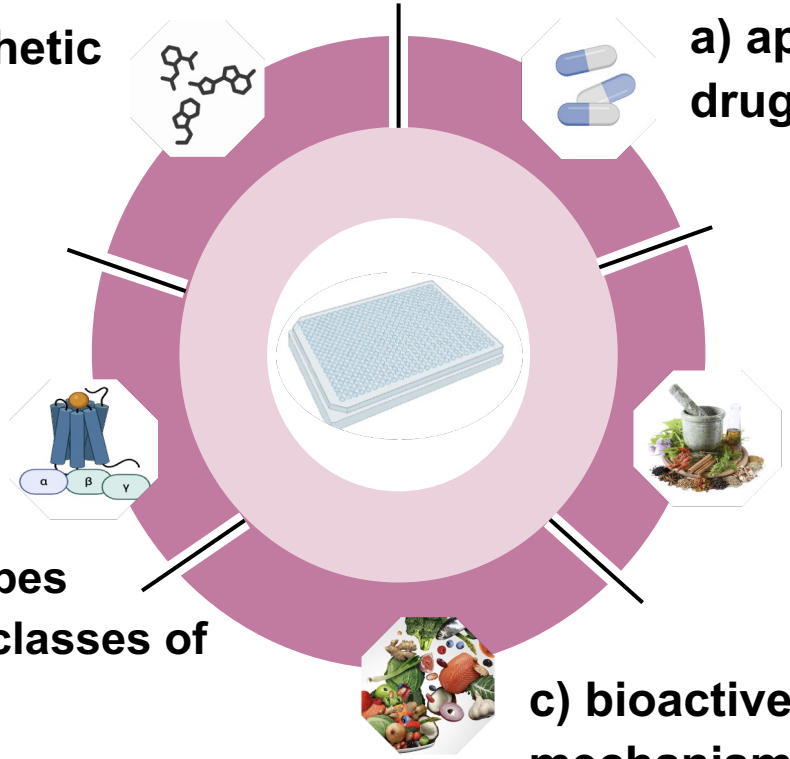
Hit to lead

Dang et al., 2016

How to select chemical library?

e) diverse synthetic compounds

a) approved or experimental drugs



b) natural products

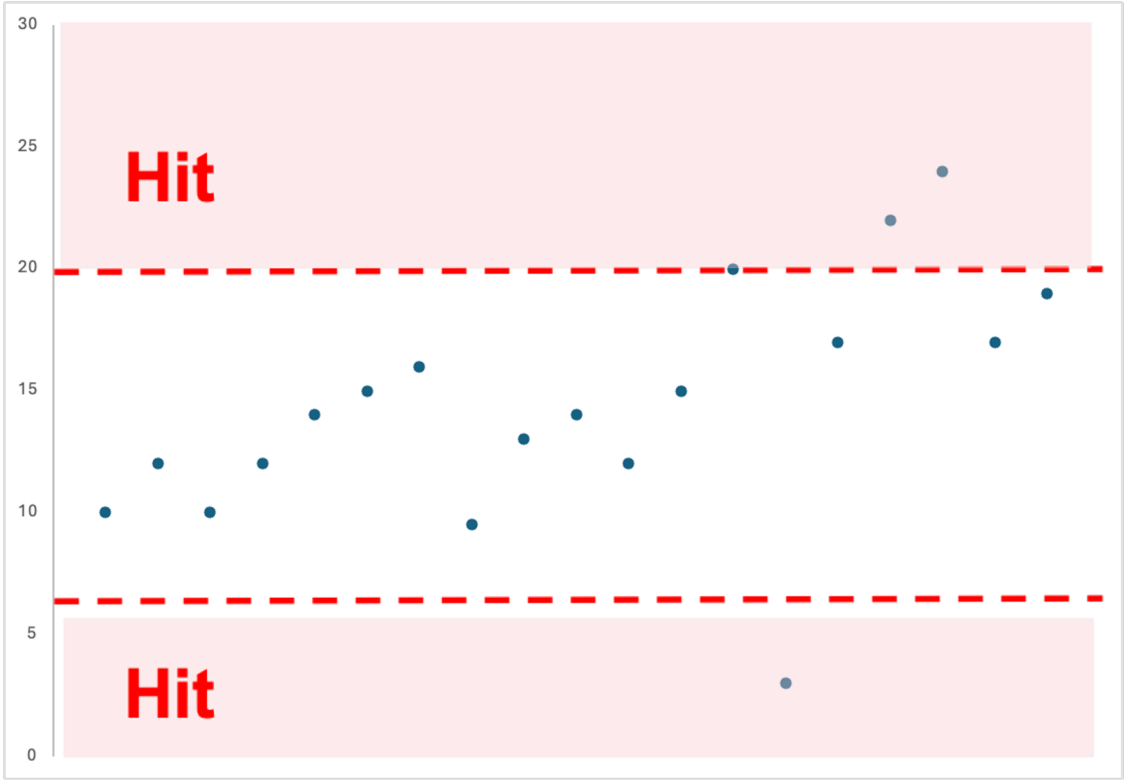
d) targeted chemotypes designed to bind to classes of proteins

c) bioactives with known mechanisms of action

Dang et al., 2016

How do researchers identify hit?

Mean ± K std (K > 3)



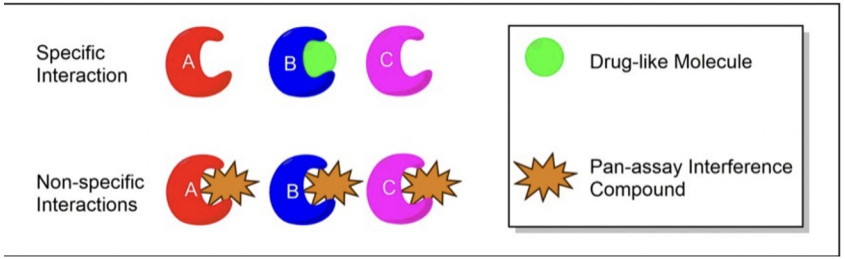
Control Group

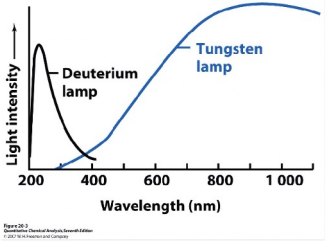
AVG	12.17
SD	2.04

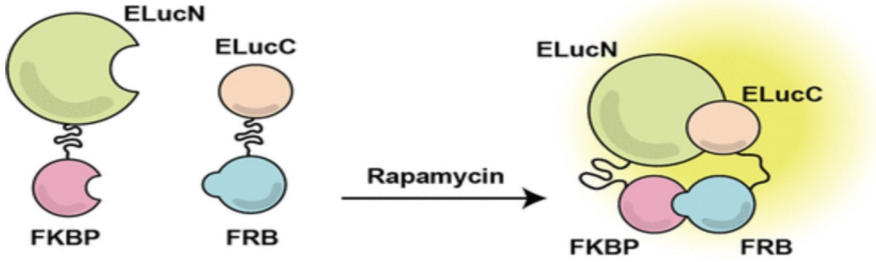
12.17 ± 3 * 2.04 = 12.17±6.12

Dang et al., 2016

How to distinguish between positive hit and false positive?







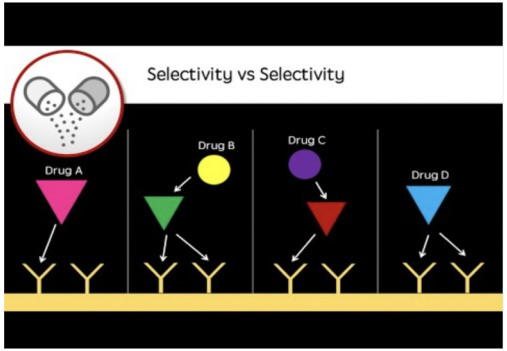
Orthogonal assay

Using a different reporter or assay format

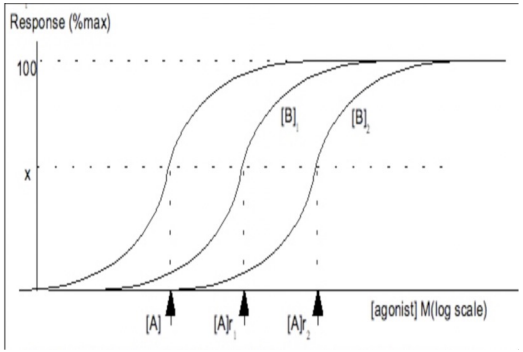
Compound interference are common causes of false positive

Thorne et al., 2010

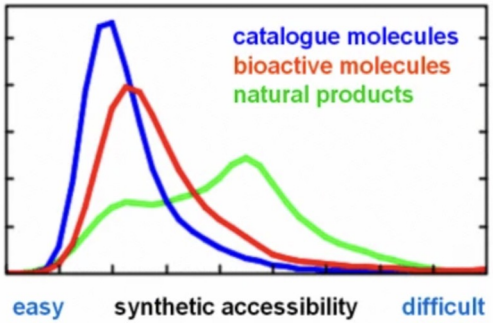
How to select hit for optimization?



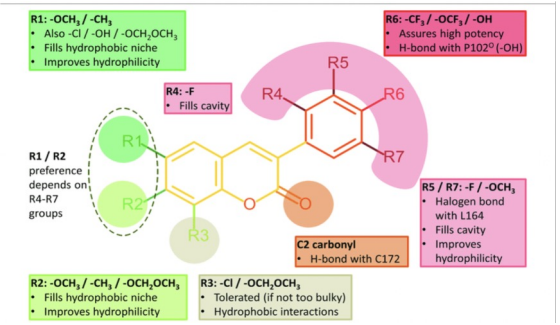
High Selectivity



Dose response curve

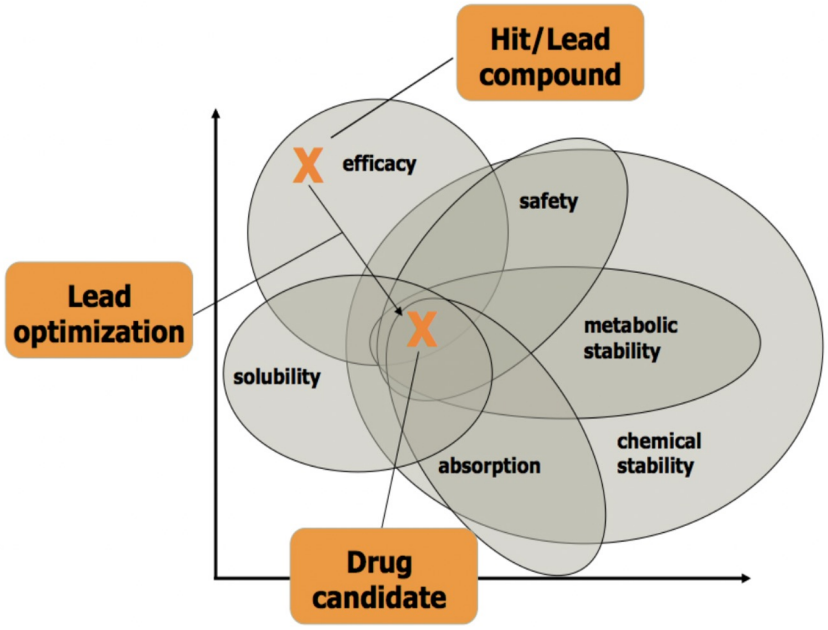


Synthetic tractability

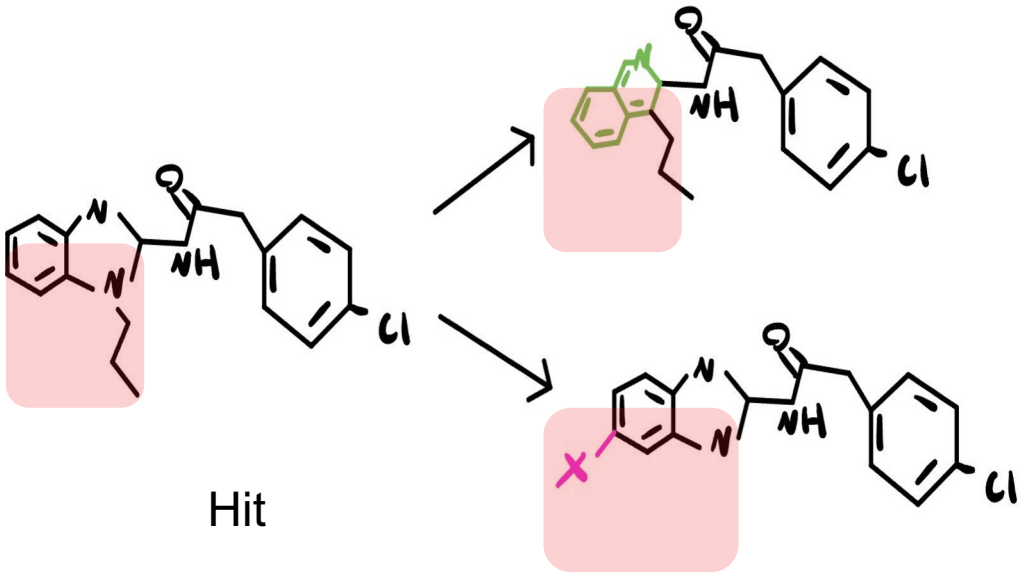


Structure-Activity Relationship

Positive hits are optimized by changing chemical structures



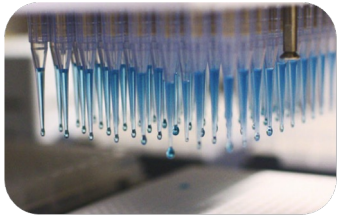
Hit to Lead Optimization

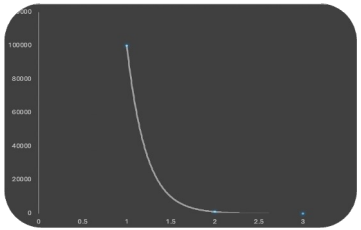


Hit

Number of compounds for each stage

Summary





HTS is an effective way to accelerate the drug discovery process and small molecule drugs are popular candidates on the discovery processes

Chemical screening contains two pathway, either pathways and assays chosen for discovery are based on special purposes

HTS method has low hit rate, but compared with traditional screening, it’s more efficient especially for large scale screening

QUESTIONS?

About the Author

Dr. Chetana Sachidanandan

The Sachidanandan lab aims to investigate neurodevelopmental disorders based on zebrafish:
Mendelian disorders,
Complex disorders

The Sachidanandan Lab

Contents lists available at ScienceDirect

European Journal of Medical Genetics

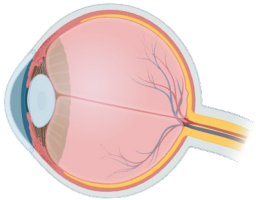
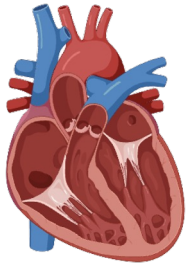
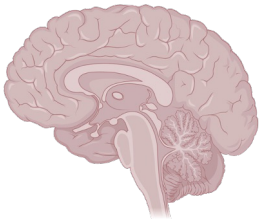
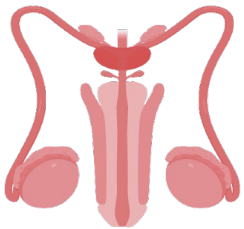
journal homepage: www.elsevier.com/locate/ejmg

Chemical screens in a zebrafish model of CHARGE syndrome identifies small molecules that ameliorate disease-like phenotypes in embryo

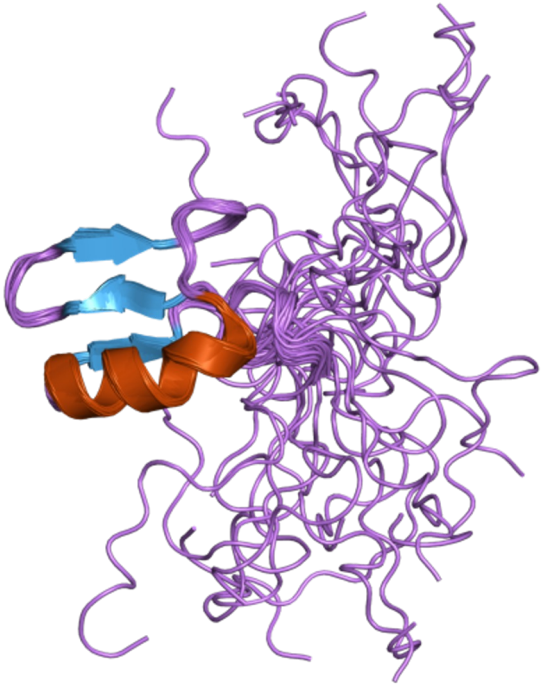
Zainab Asad[a,b,1], Chetana Sachidanandan[a,b,*]

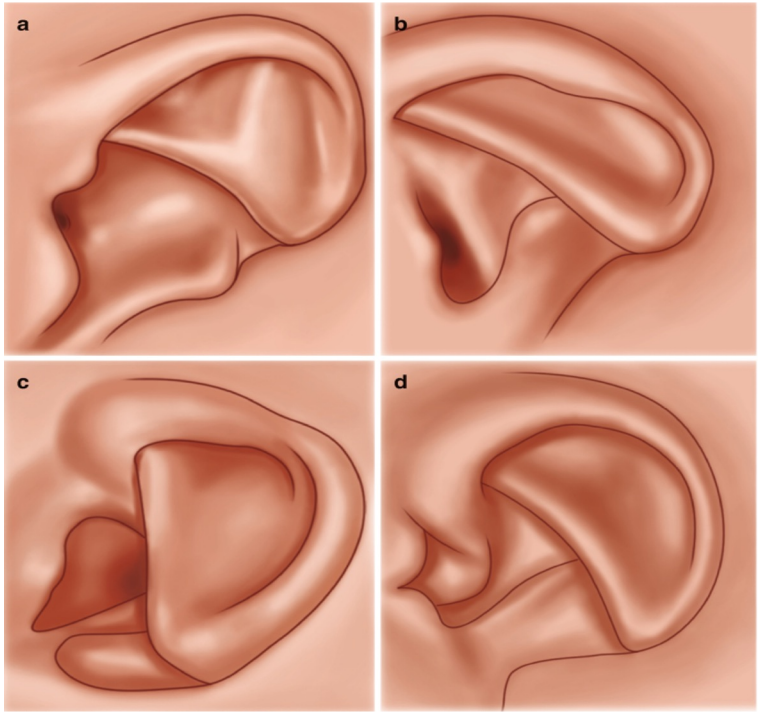
[a] CSIR-Institute of Genomics and Integrative Biology (CSIR-IGIB), New Delhi, 110025, India
[b] Academy of Scientific and Innovative Research (AcSIR), New Delhi, India

What are the symptoms of CHARGE syndrome?

C	H	A	R	G	E
Coloboma Of eye	Heart defects	Atresia of choanae	Retardation of growth	Genital hypoplasia	Ear anomalies
					

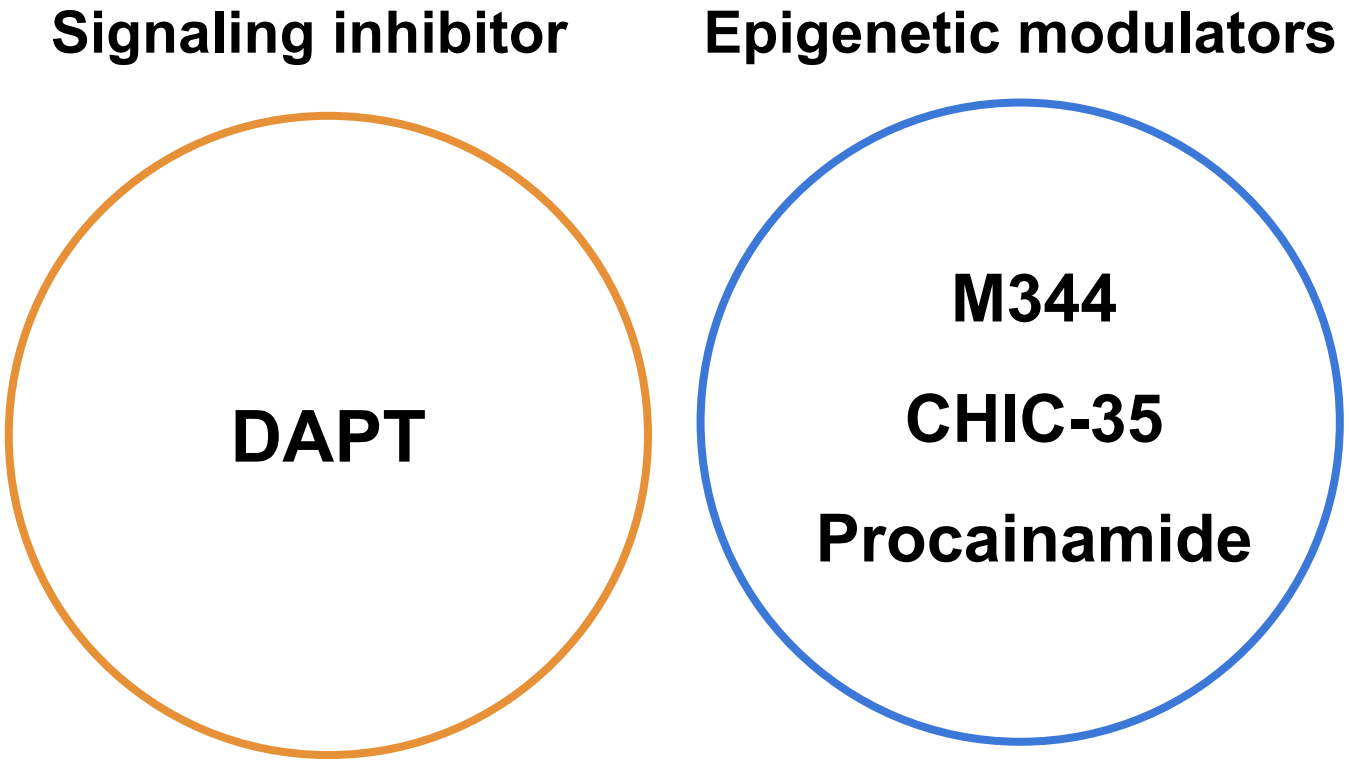
CHD7 is associated with CHARGE syndrome



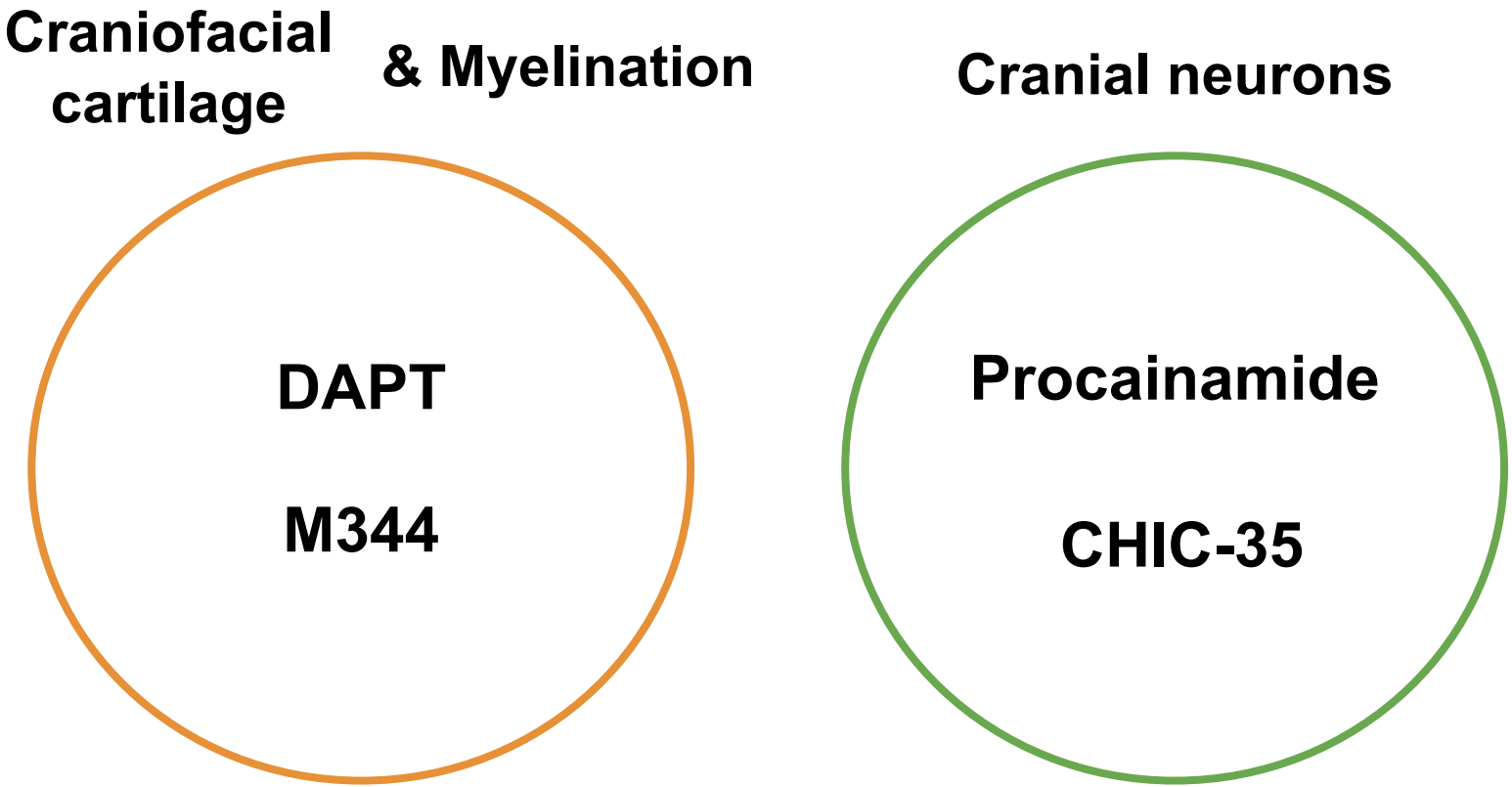


CHD7 - Chromodomain helicase DNA binding protein 7, found in body parts such as eye, ear, and brain during embryonic stage

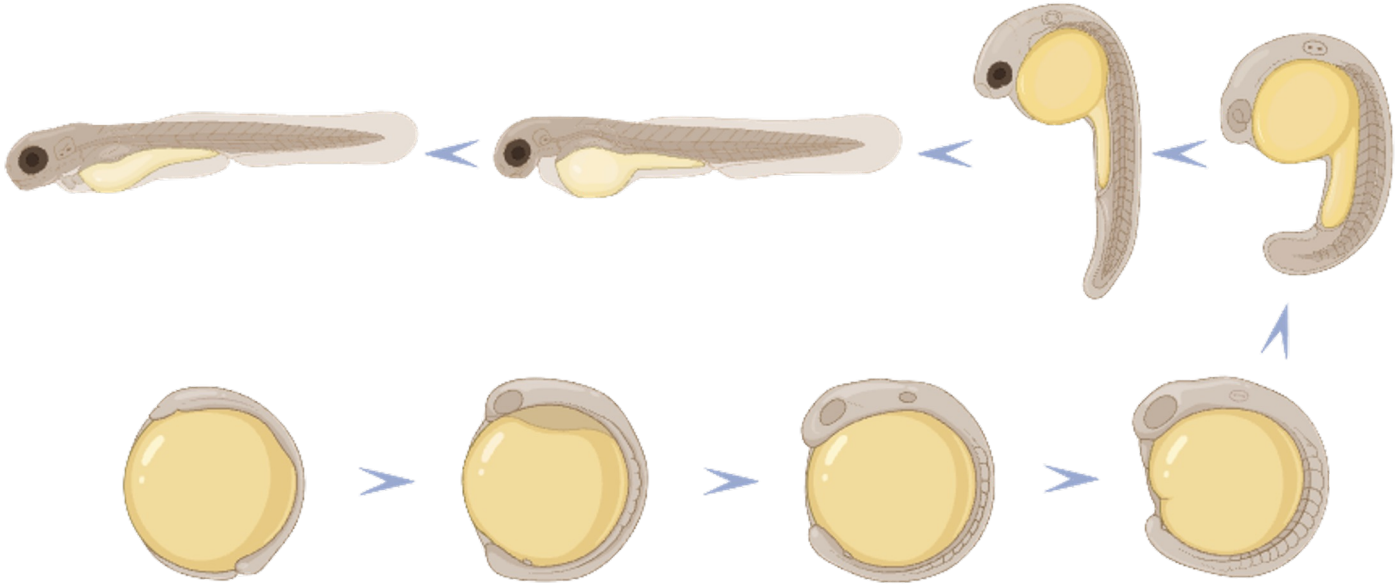
Which chemical compounds were known to target CHD7?



Which chemical compounds were known to target CHD7?



Why use zebrafish as the model organism?



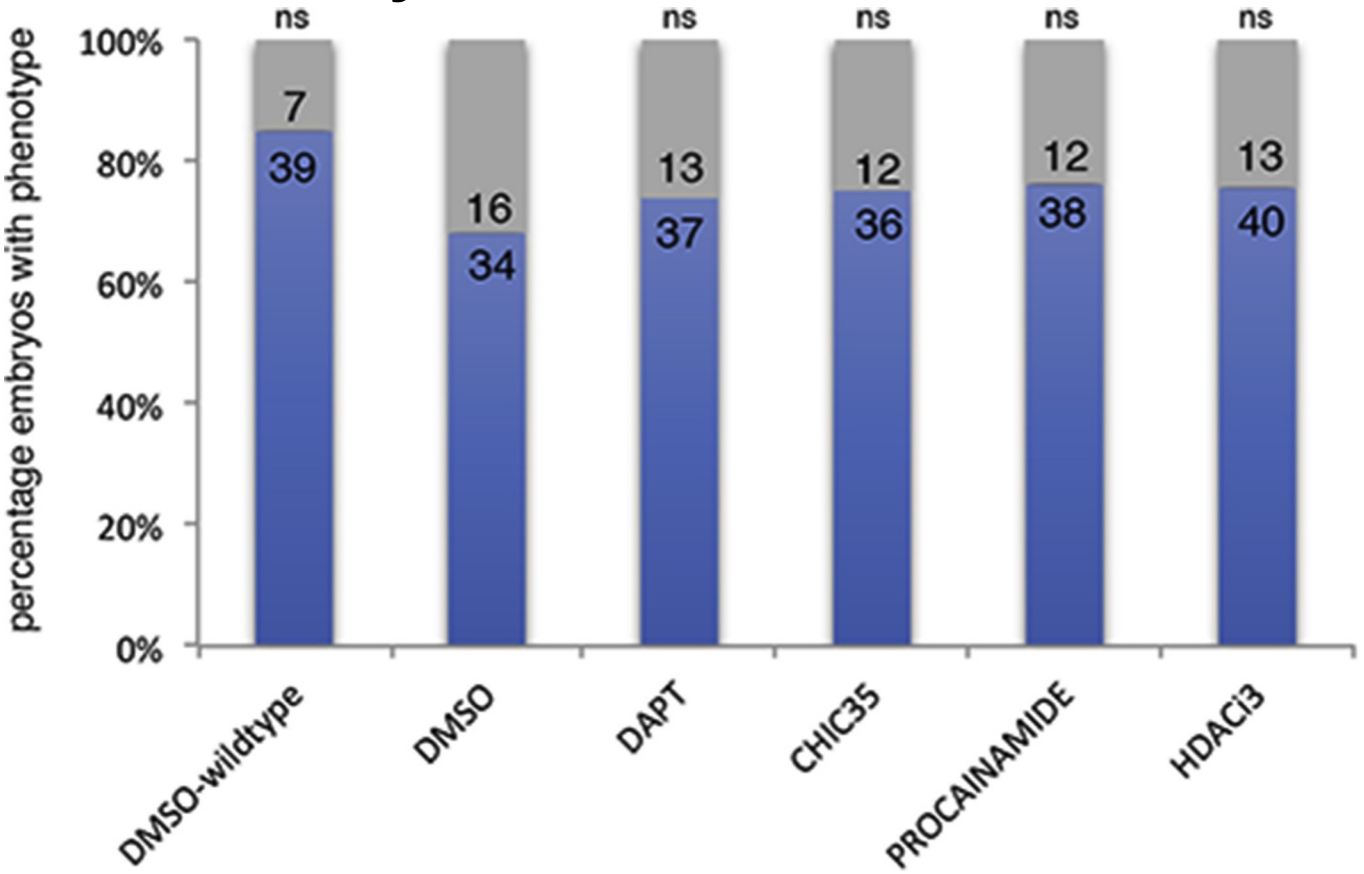
Similar disease-associated gene

Transparent embryo

Rapid development

Similar drug metabolism pathway

How did they decide the concentration of compounds?



5μM of each compounds were used, no significance in the survival of embryos.

Figs. 1 Survival rate of embryo with different compounds

How does knockdown of CHD7 recapitulate CHARGE syndrome?

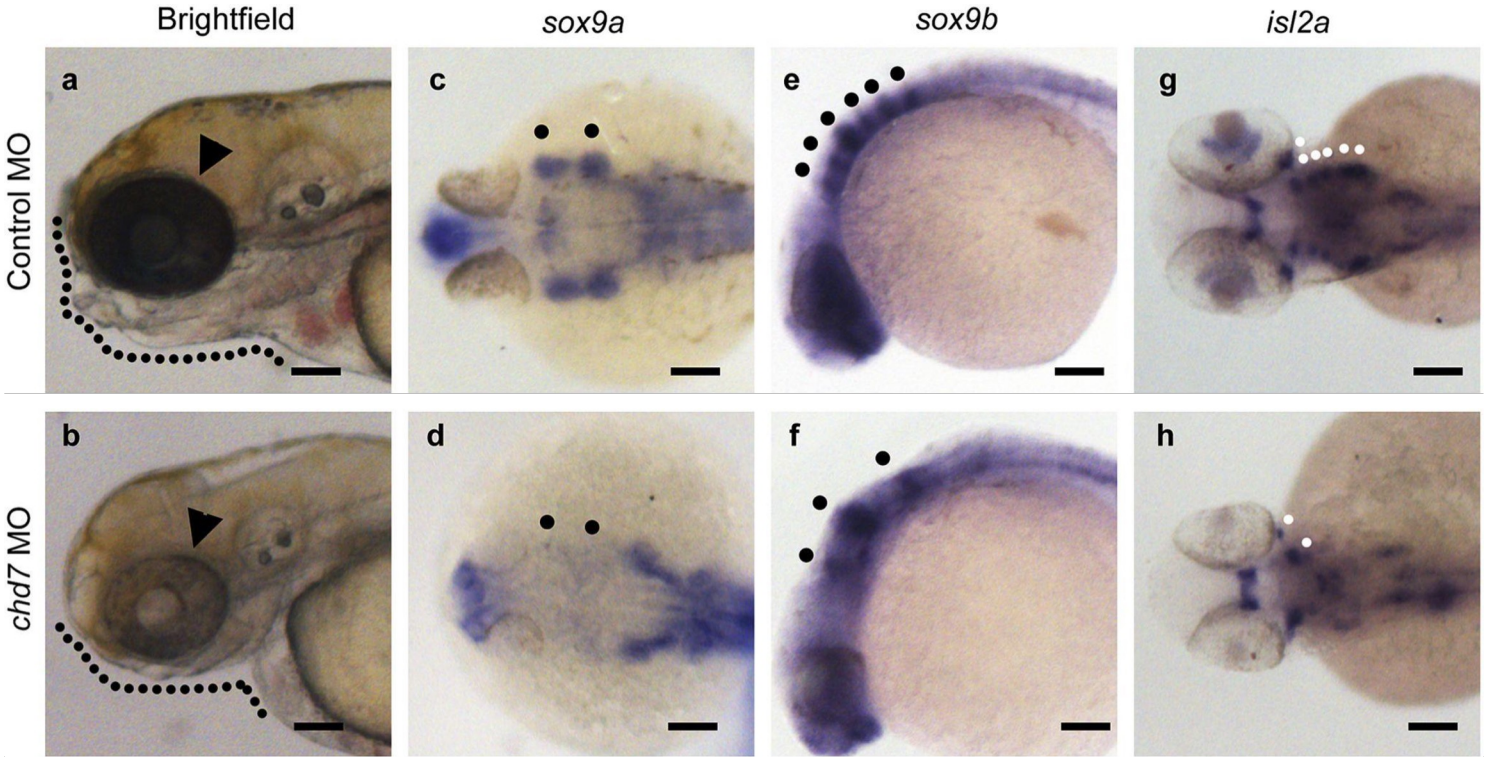


Fig 1a,c,e,g. Control group

Fig 1b,d,f,h. Treatment group

The treatment group displayed physical changes compare to the control group

How does knockdown of CHD7 recapitulate CHARGE syndrome?

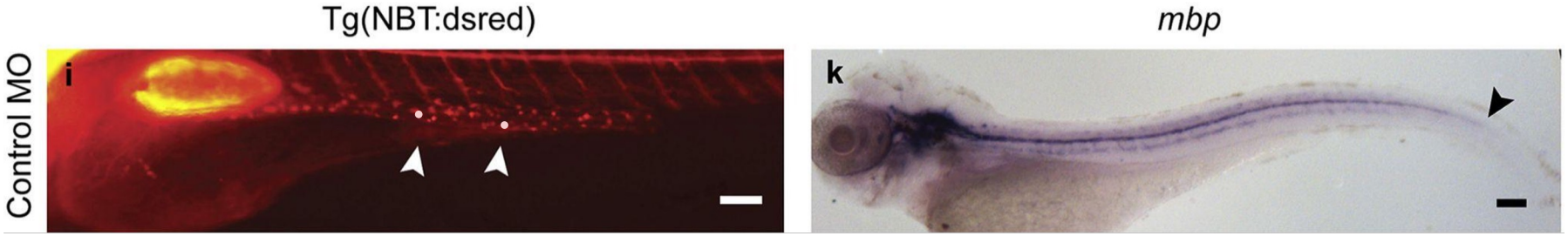


Fig. 1i, k Controlled enteric neurons and glial cells at 4 dpf

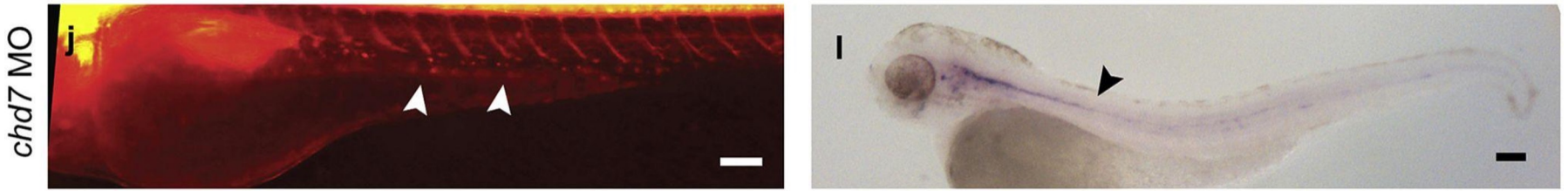
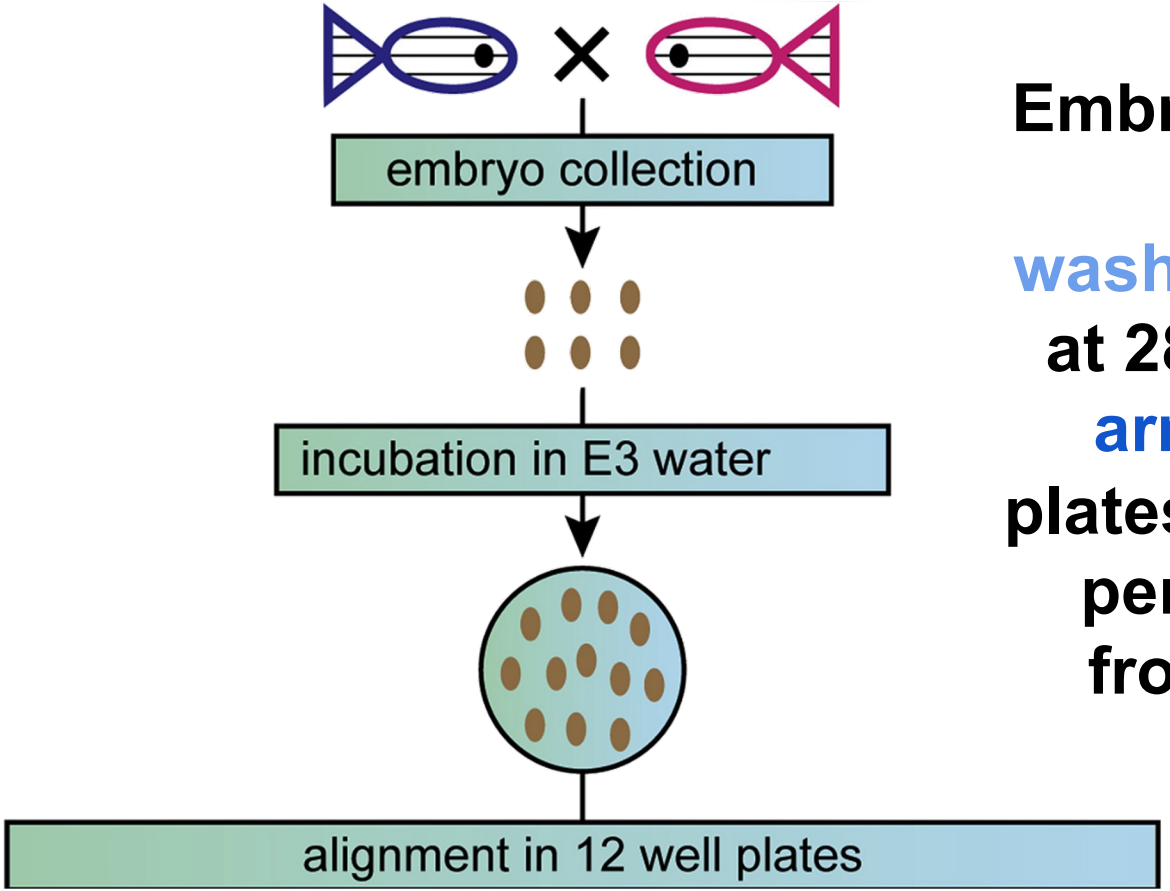


Fig. 1j, l Treated enteric neurons and glial cells at 4 dpf

CHD7 morphants have severe reduction in enteric neurons and glial cells

How they perform chemical screens on zebrafish embryos?

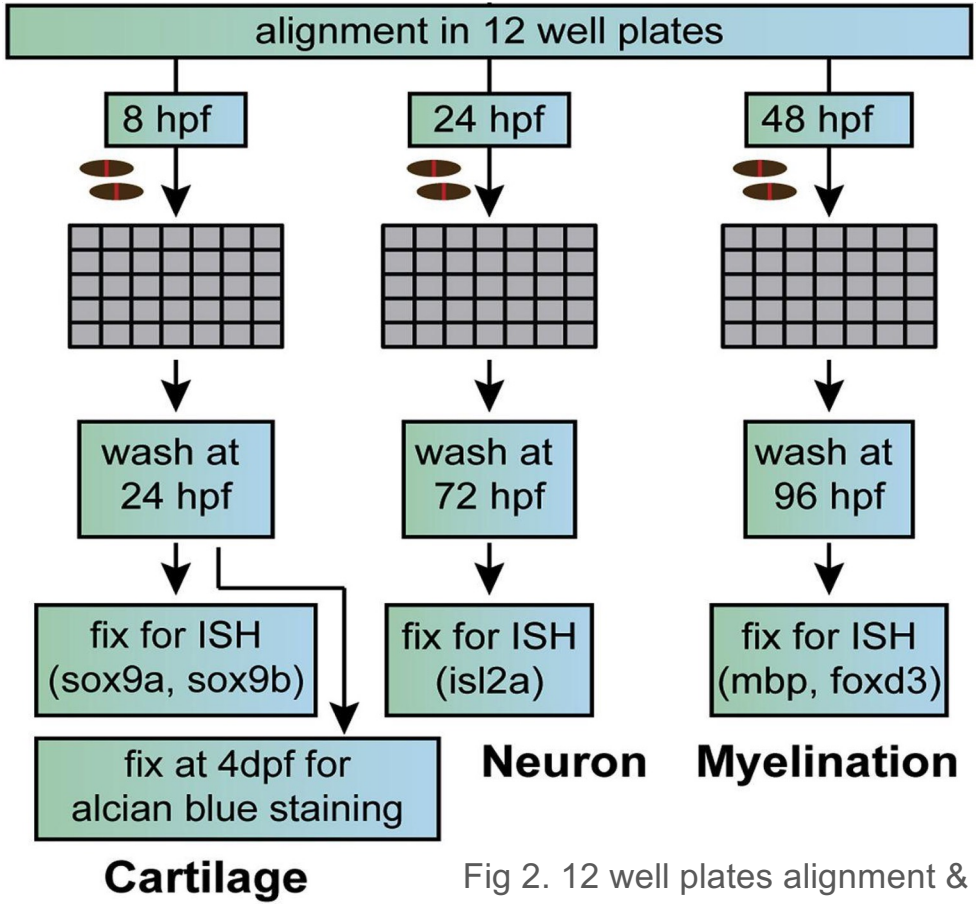


Embryo collected after fertilization, washed and incubated at 28ºC in egg water, arrayed in 12 well plates with 25 embryos per well, observed from 1dpf to 4 dpf

dpf: days post fertilization

Fig 2. Embryo collection & incubation

How they perform chemical screens on zebrafish embryos?



Cartilage lineage: alcian blue staining

Neuronal lineage: fluorescent imaging

Myelination lineage: Schwann cell marker staining

Fig 2. 12 well plates alignment & staining

In CHD7 morphants, which compounds can recover jaw structures?

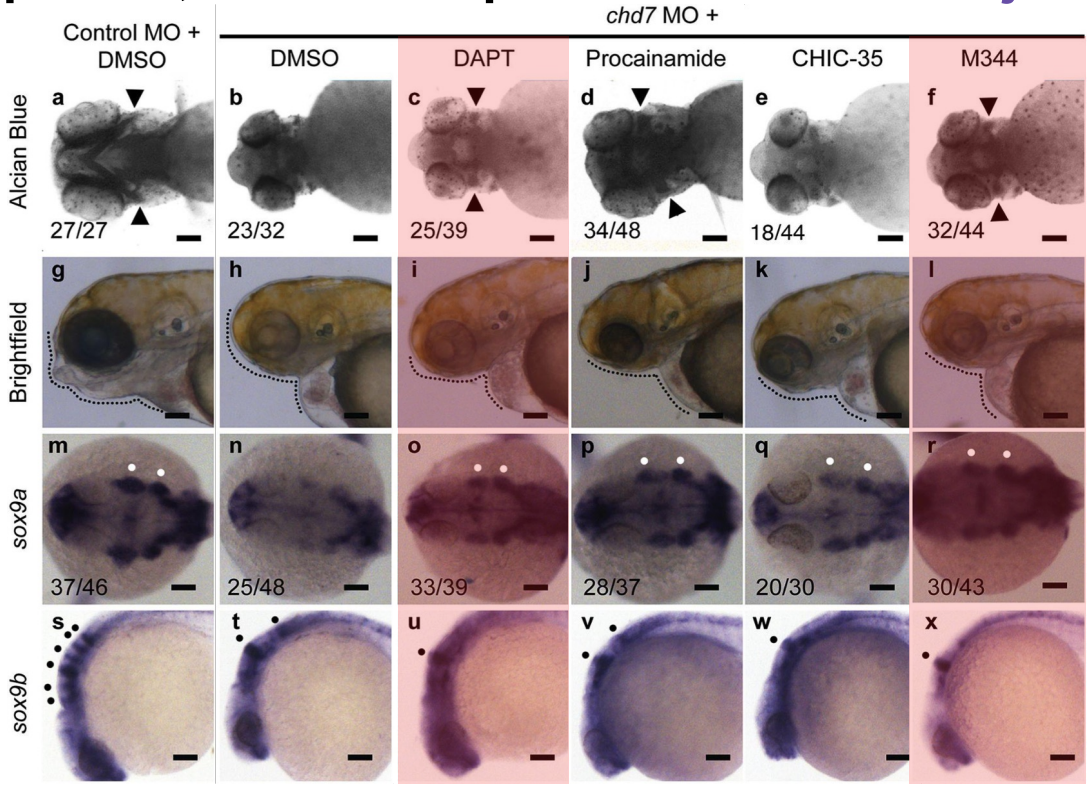
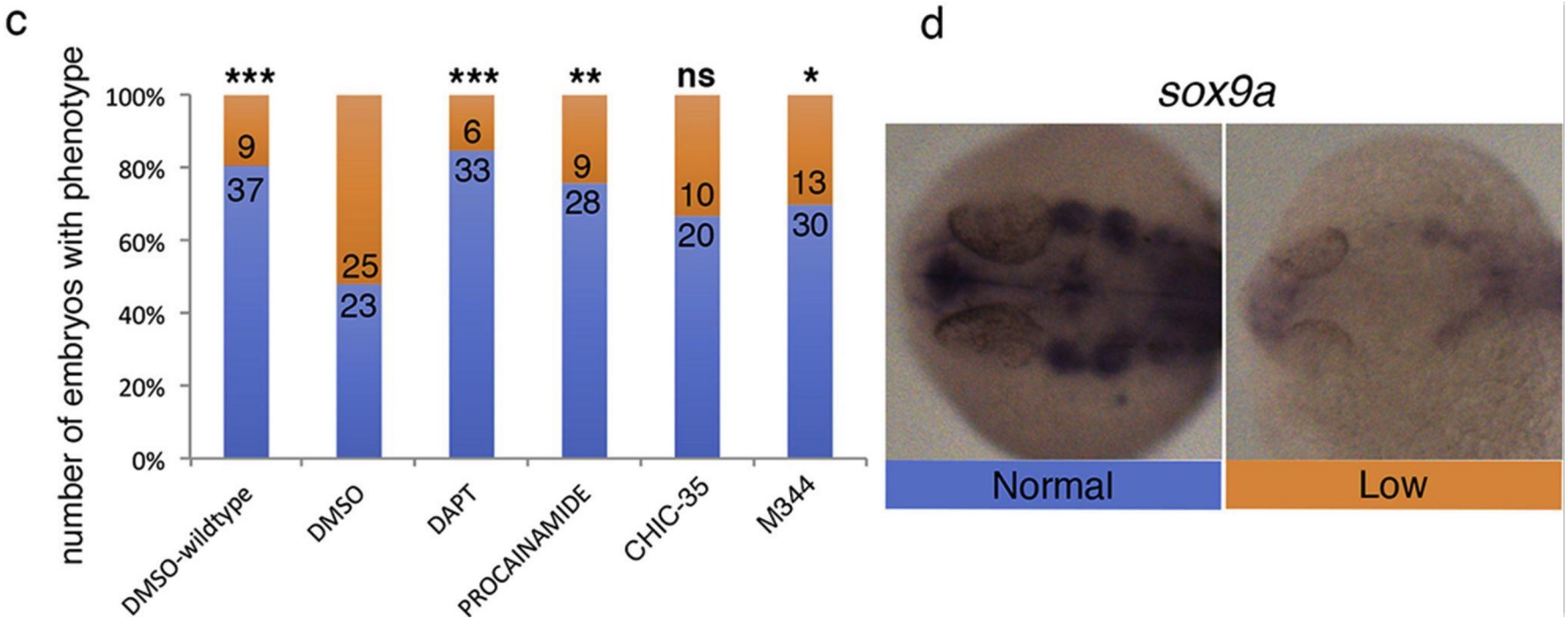


Fig. 3 Jaw structures of control and treatment

The 4 compounds recover jaw structures to different extent, but did not recover the sox9b expression

In CHD7 morphants, which compounds can recover jaw structures?



Supplement Fig. 2 Insignificant (p>0.05), *(p≤ 0.05), **(p ≤0.01), ***(p≤ 0.001), ****(p≤0.0001)

There is a significant reduction in embryos lacking cartilage staining when treated with the four compounds

Which compounds rescue cranial neurons?

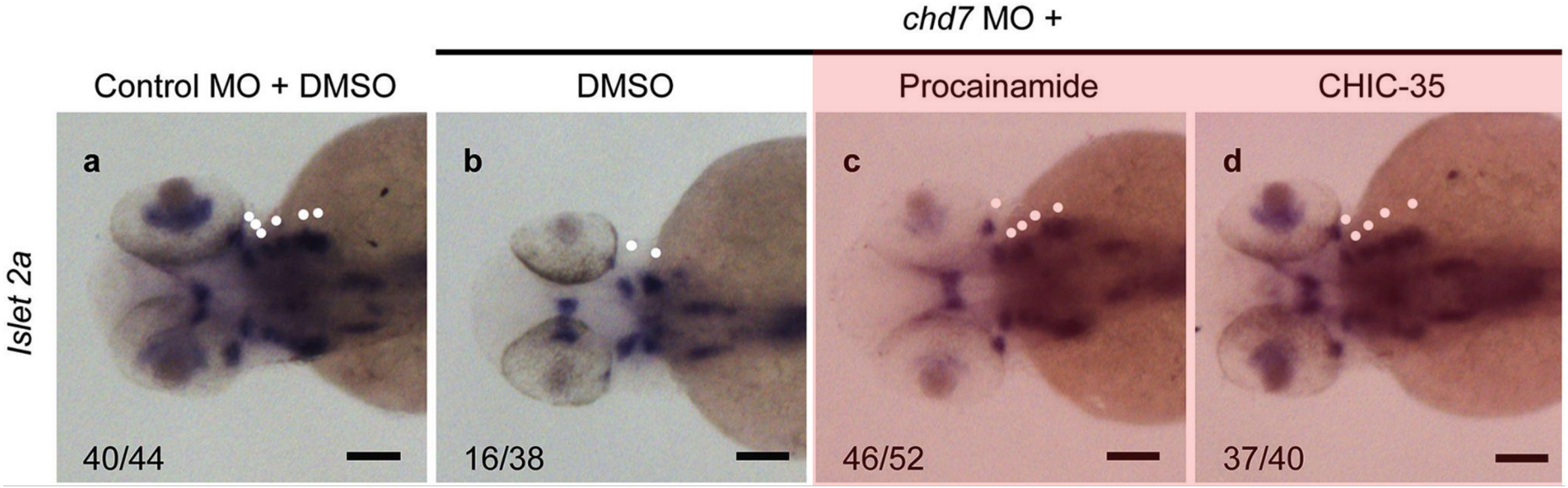


Fig. 4 Sensory and motor neurons at 72 hpf

Procainamide and CHIC-35 can partially rescue defects in cranial neurons

Which compounds recover enteric neurons?

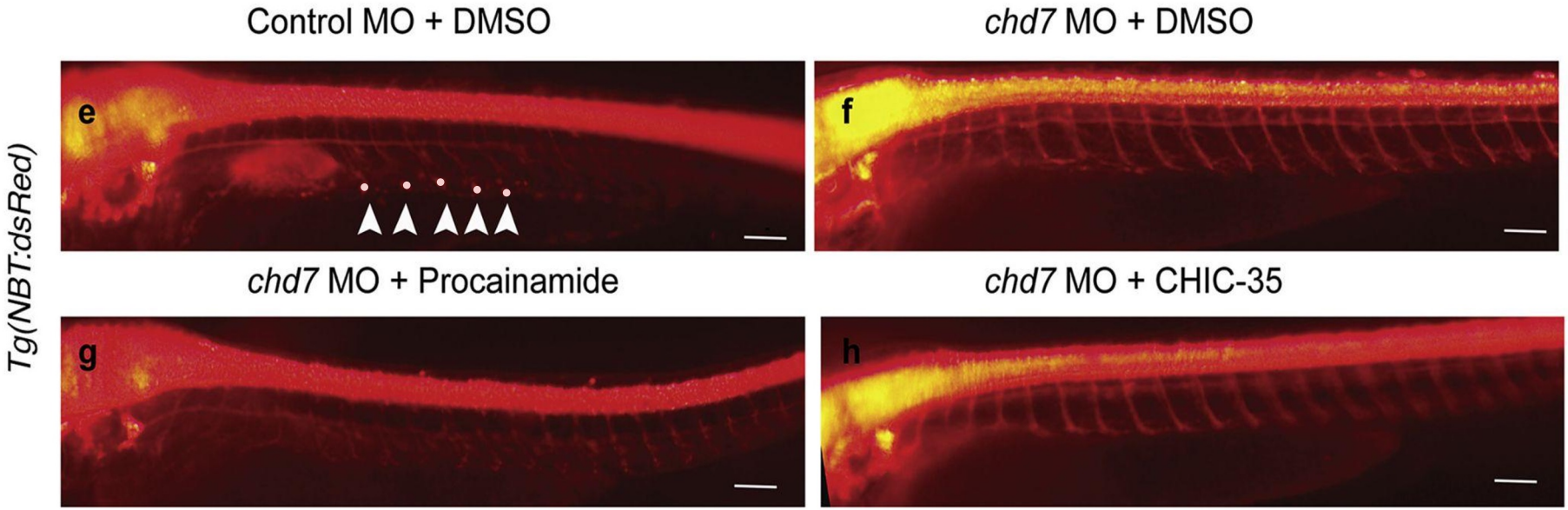


Fig. 4 Enteric neurons at 4dpf

Treatment with Procainamide of CHIC-35 did not induce recovery of enteric neurons

Which compounds rescue myelination?

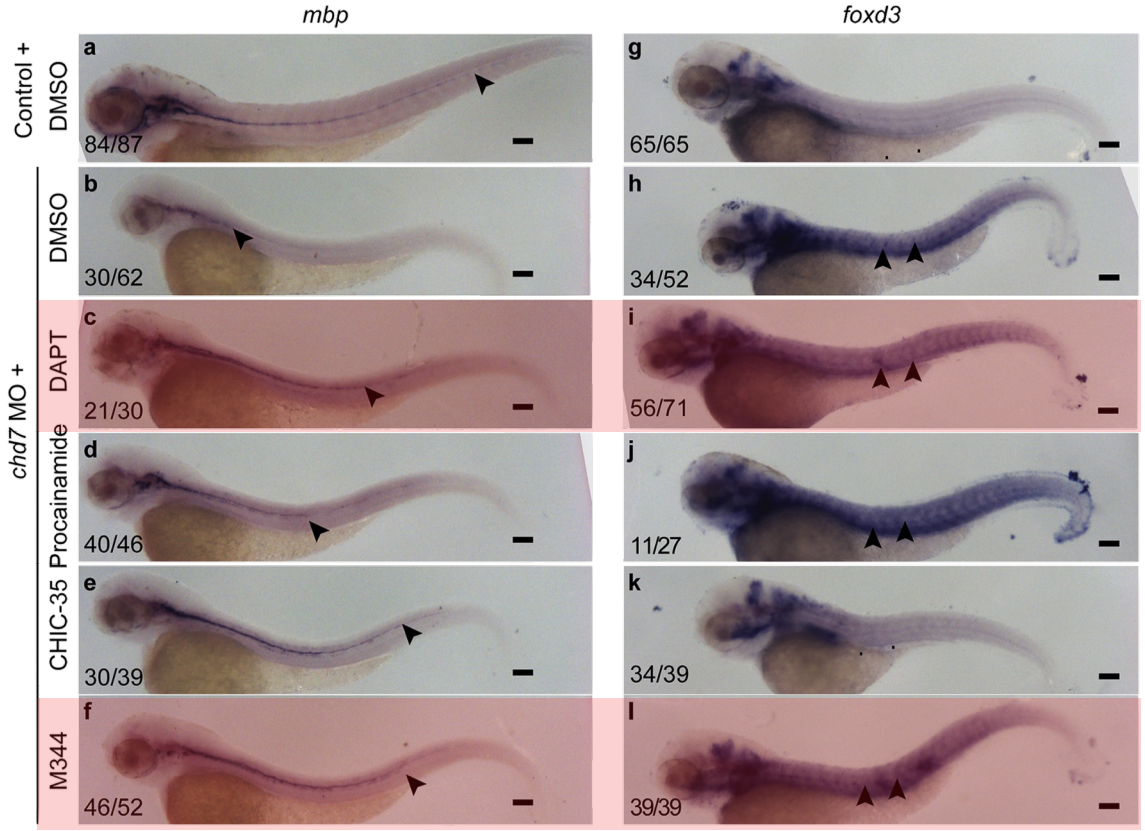


Fig. 5 Rescue of myelination defects at 4dpf

Different compounds rescue myelination to different degrees

Which compounds rescue myelination?

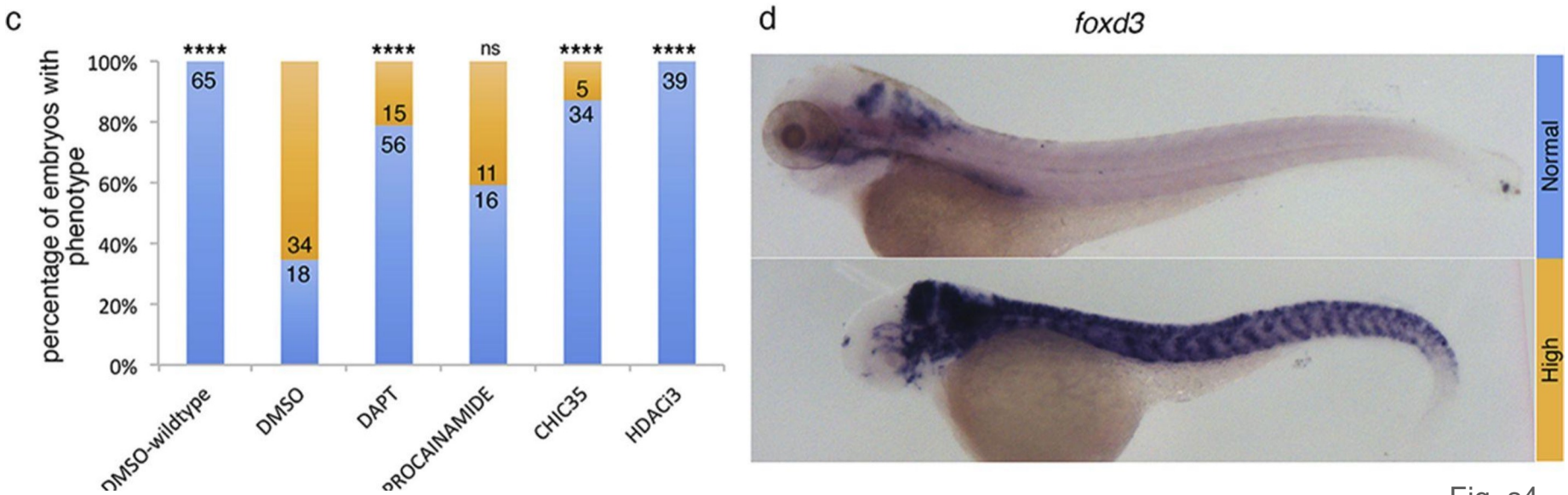
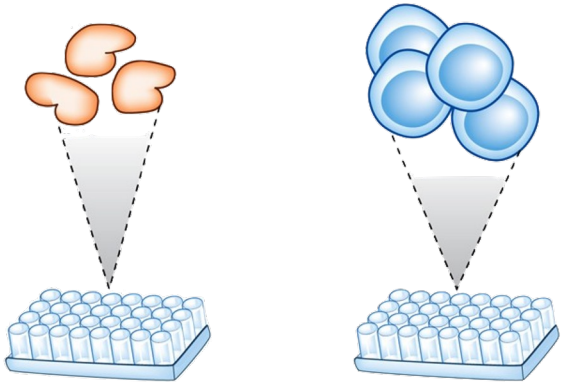


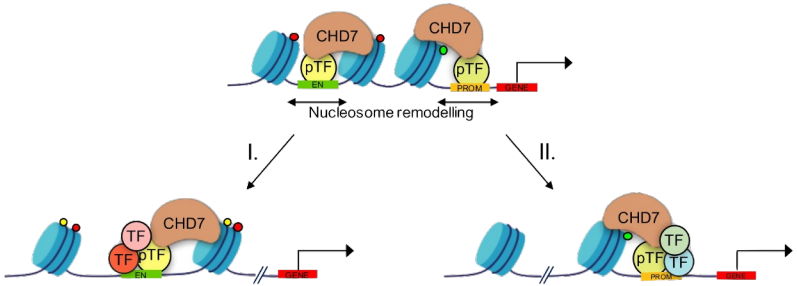
Fig. s4

There is significant reduction in embryo lacking myelination staining

What is the future direction of this study?



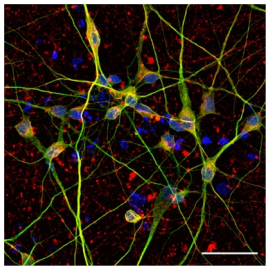
Discover a single compound that ameliorates or reverses all the phenotypes



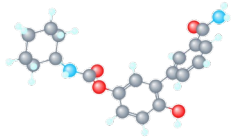
Look into the CHD7 chromatin remodeling activity

Summary

CHD7 mutation disrupts gene expression during embryonic stage



4 compounds, DAPT, M344, CHIC-35, Procainamide, were identified through chemical screening to rescue embryos from disease-like phenotypes



Small molecule compounds may be the key to solve CHARGE syndrome

QUESTIONS?


THANK
YOU

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