Functional genomics of the prion life cycle

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(feel free to take pics, tweet & share as you see fit)§

Work performed by:

- Vangelis Bouris
- Davide Caredio
- Elena De Cecco
- Sebastian Hachenberg
- Tingting Liu
- Carlos Oueslati Morales
- Sandesh Neupane
- Stefano Sellitto
- Chiara Trevisan
- Dalila Laura Vena
- Hao Wang
- Yancheng Wu
- Jiang-An Yin

The rationale for unbiased genetic perturbations

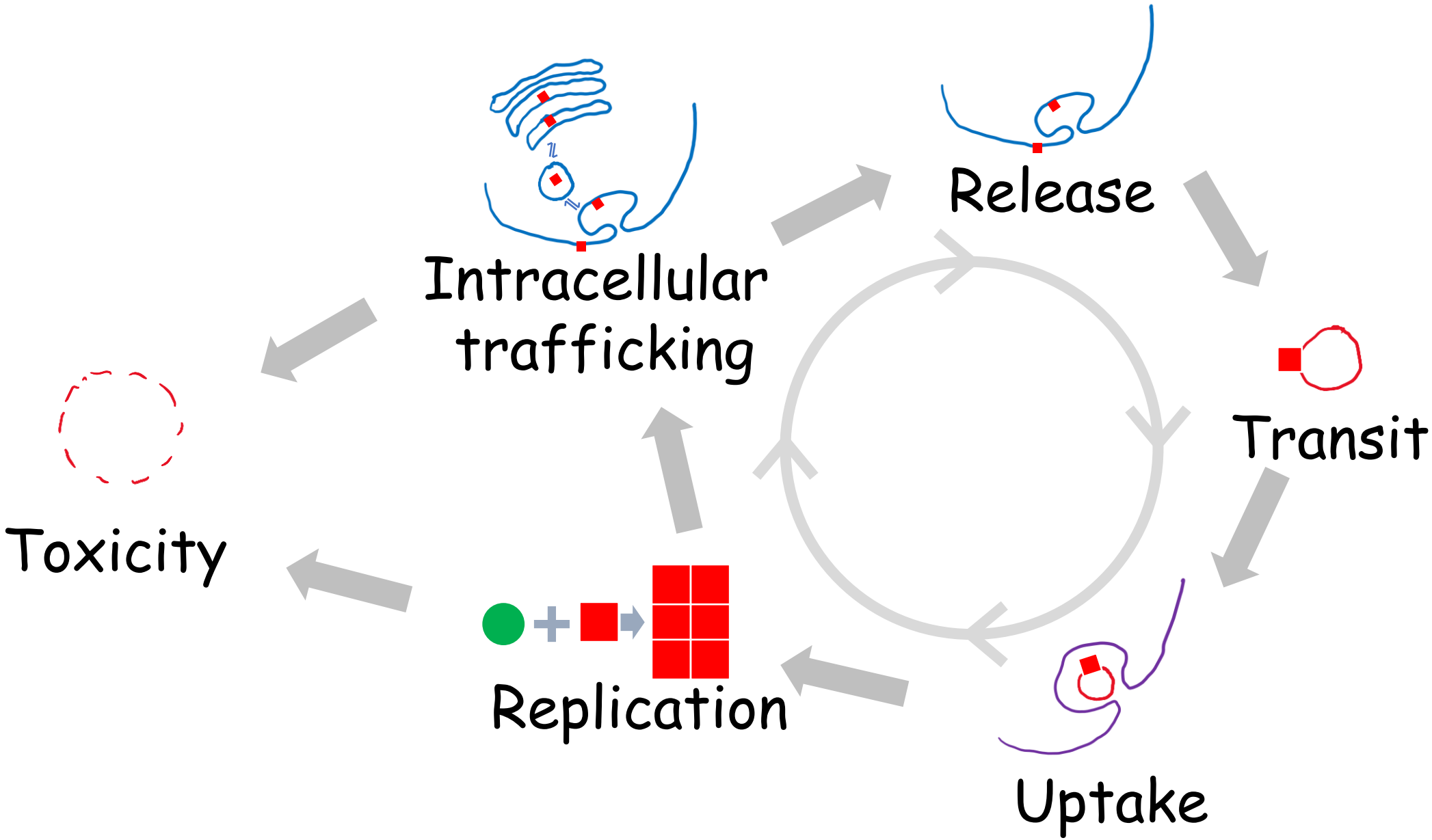
- Therapy of neurodegeneration is held back by a dearth of actionable targets.
  - For prions, I see only PrP.
  - For AD, PD and ALS, I see ≤4 plausible targets each.

- For decades, neurodegeneration research has been hypothesis-driven. It's time to explore hypothesis-free approaches.

- Case in point: metabolic syndrome and alcohol addiction
  - 50 years of hypothesis-based lifestyle interventions haven’t yielded much
  - GLP-1 agonists came out of serendipity, and are having a huge impact

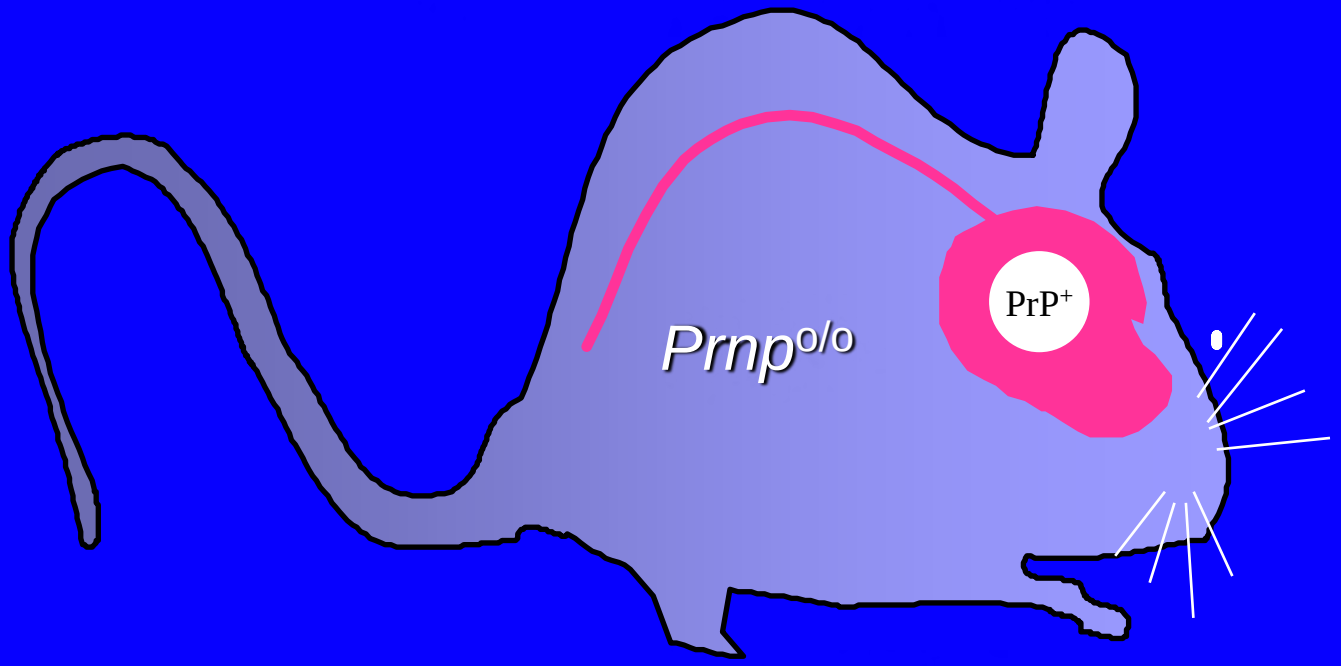
- **How can we speed up serendipity?**

The prion life cycle

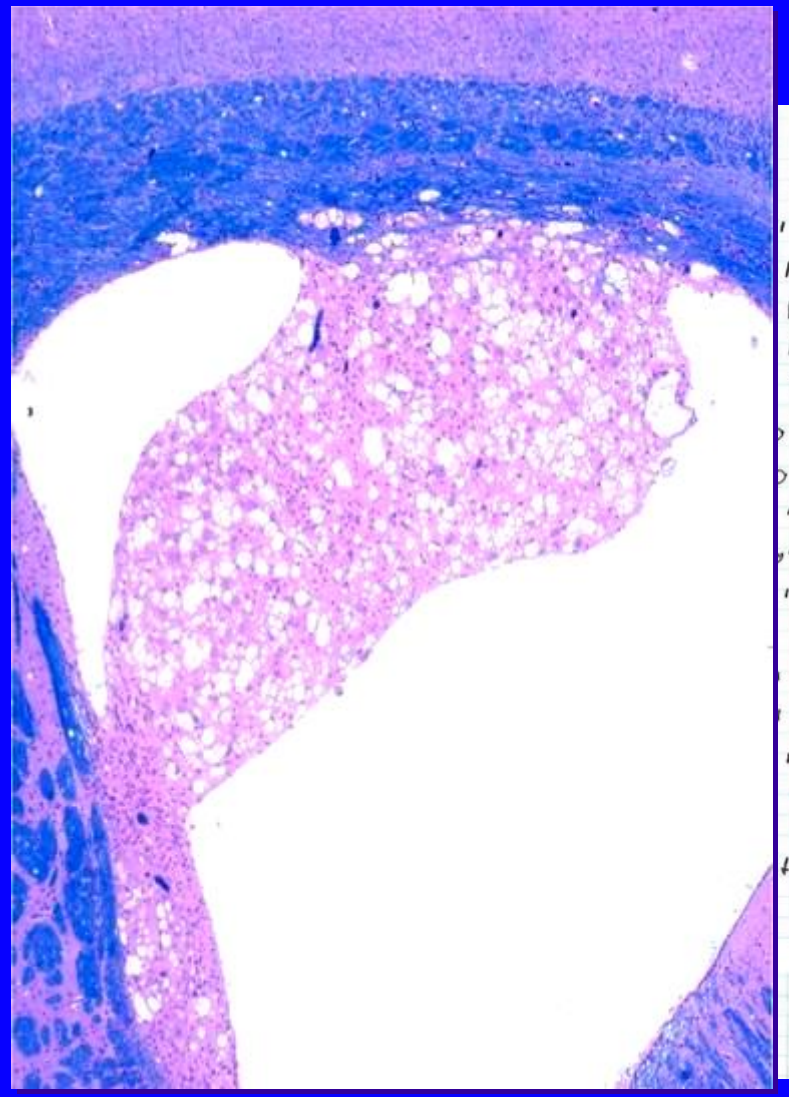


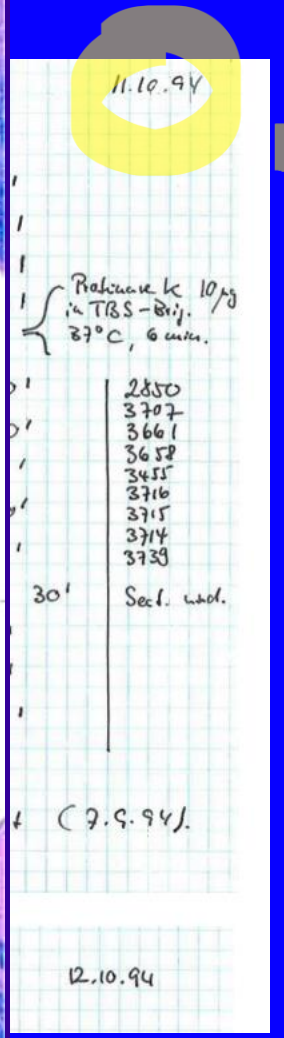
PrP^{c} transduces prion toxicity!!!

- Grafts develop prion disease
- *Prnp*$^{o/o}$ hosts stay healthy.



Brandner etc,
Nature 1996





Selective vulnerability to prions of different cell types

Some neurons

Death

Most cell lines, muscle, lymphoid tissue, etc.

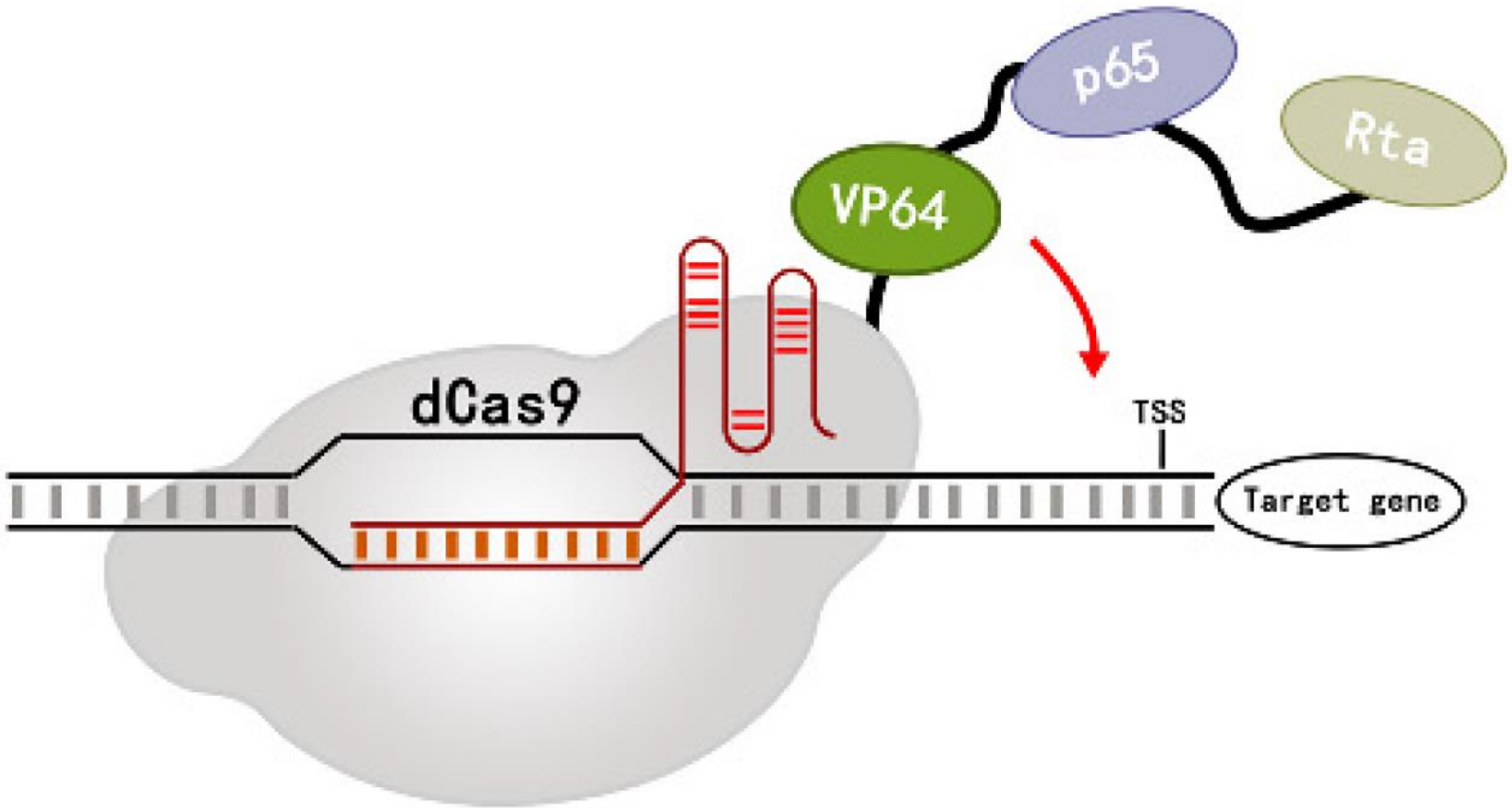
Nothing

Synthetic lethality

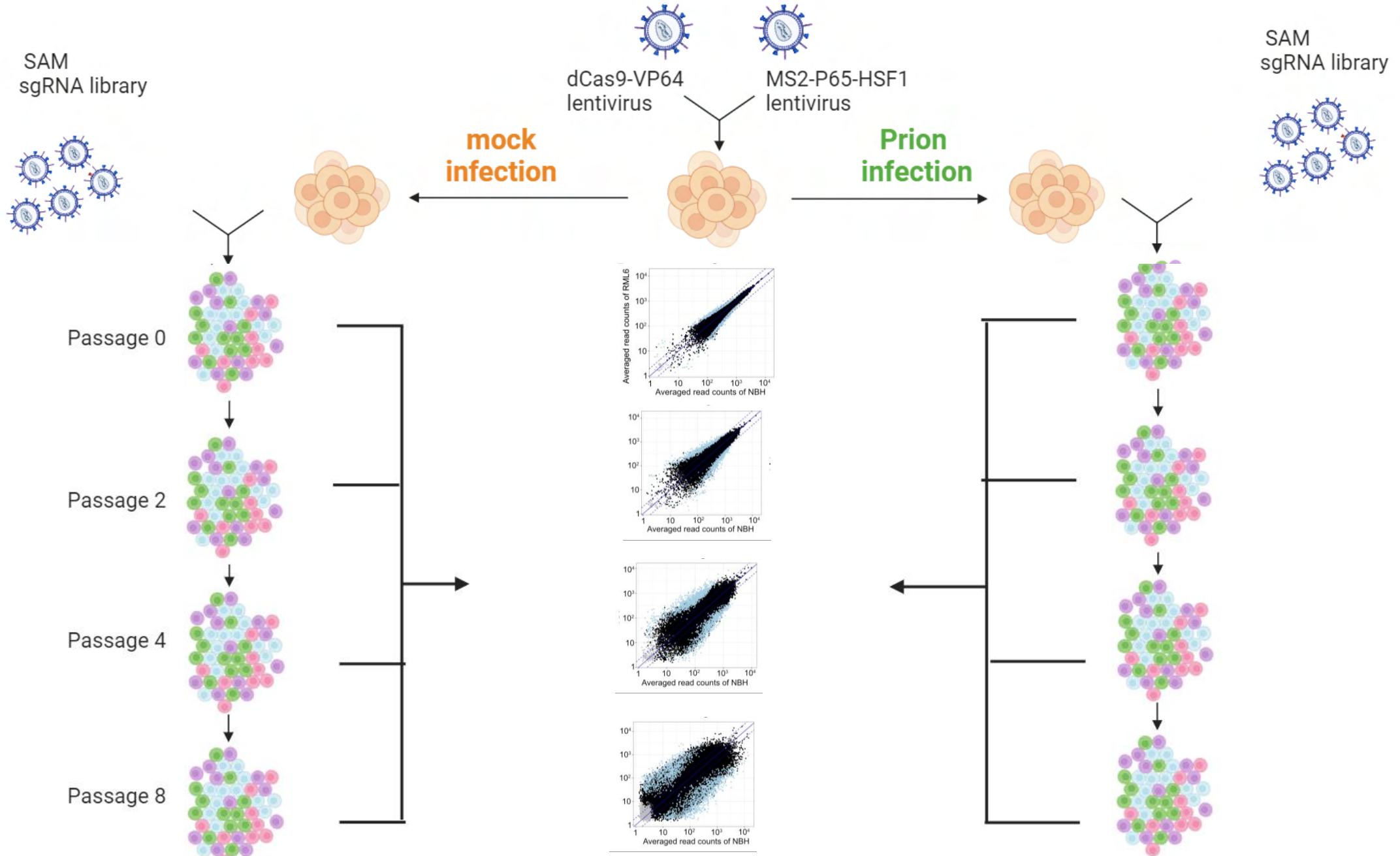
Maybe they lack some toxicity transducers?

Tingting Liu
Jiang-An Yin

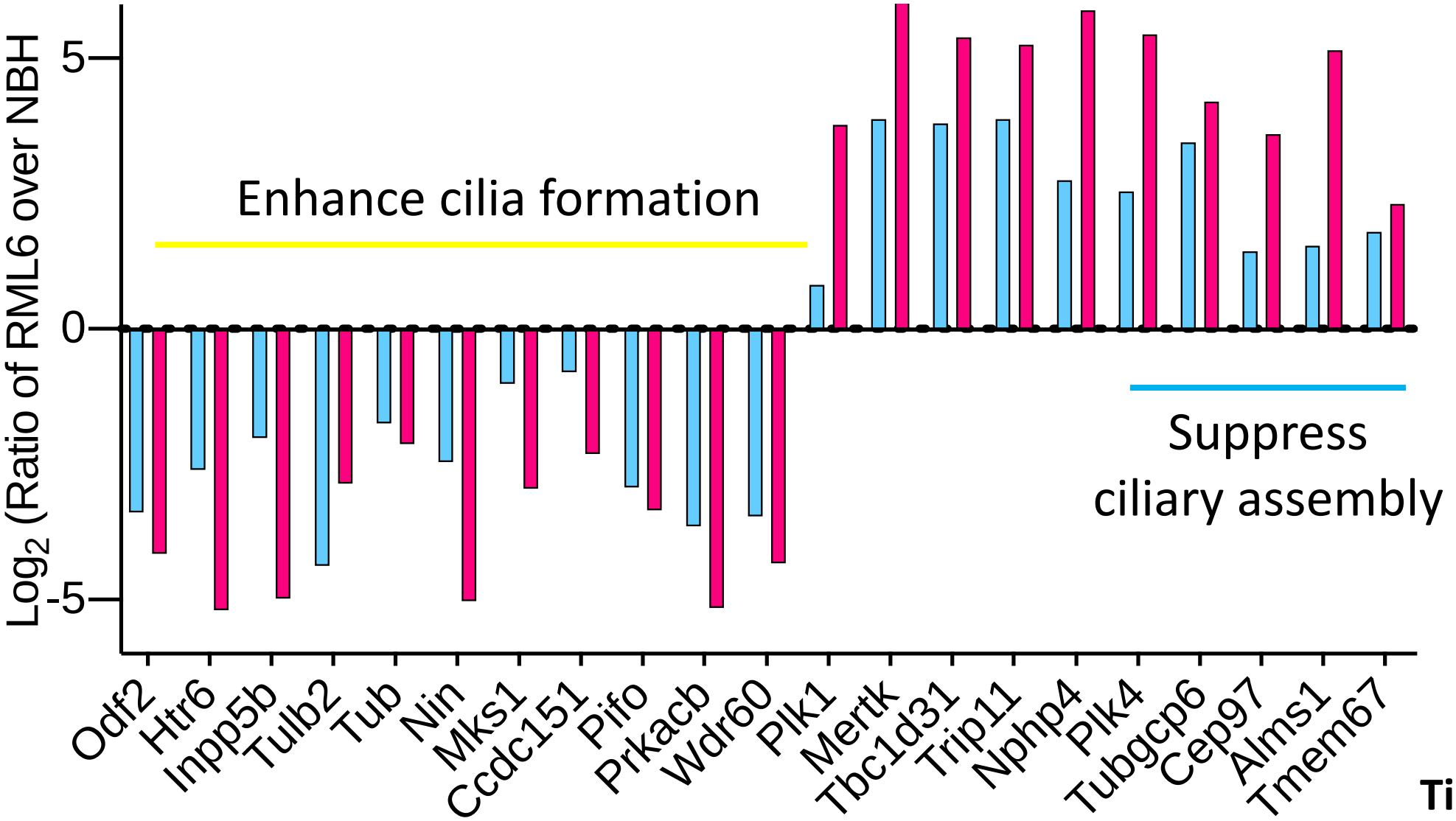
Programmable gene activation by CRISPR-a



CRISPR-activation synthetic-lethal screen for transducers of prion toxicity



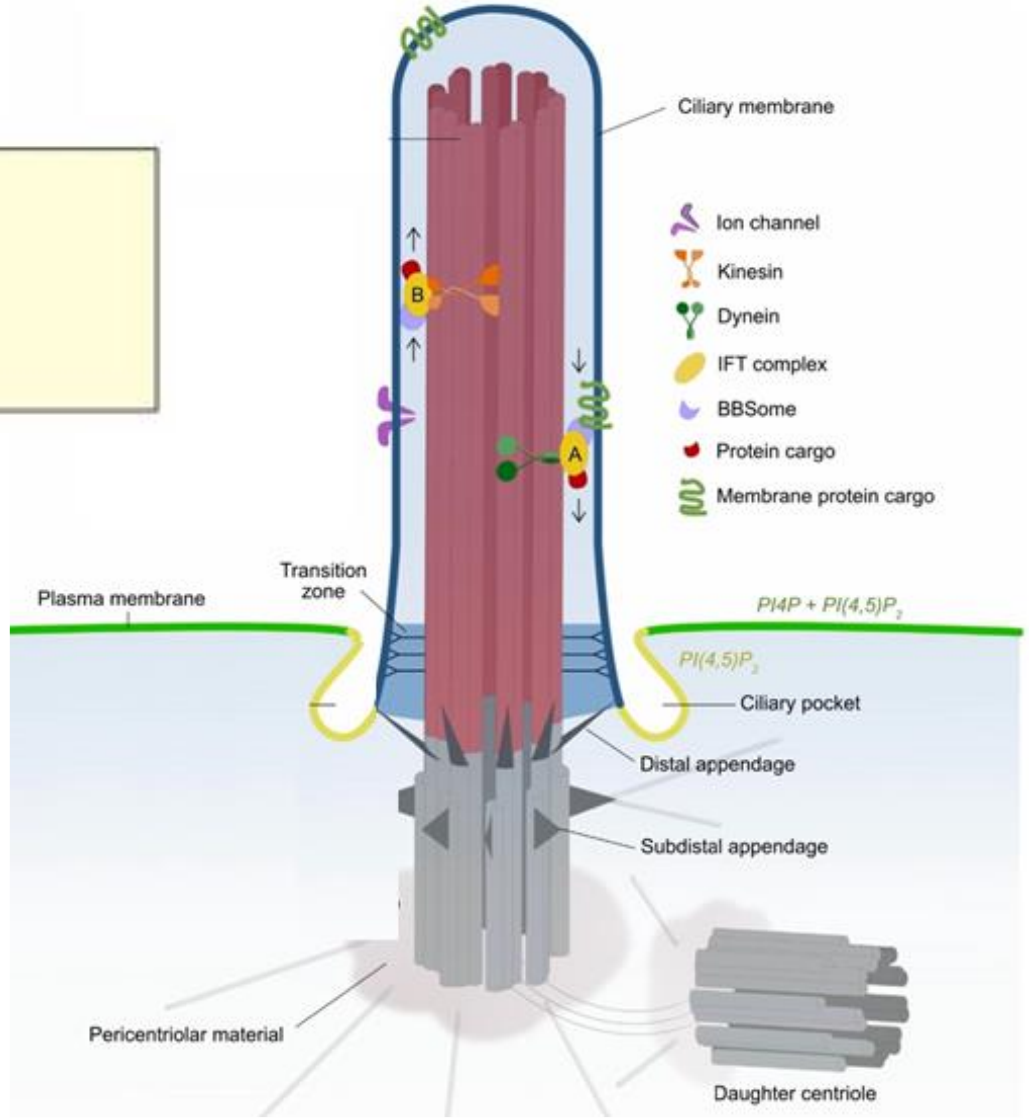
Ciliogenesis correlates with prion toxicity



Tingting Liu
Jiang-An Yin

What is the primary cilium, and why is it there?

Trafficking
IFT complexes
BBSome
Motors



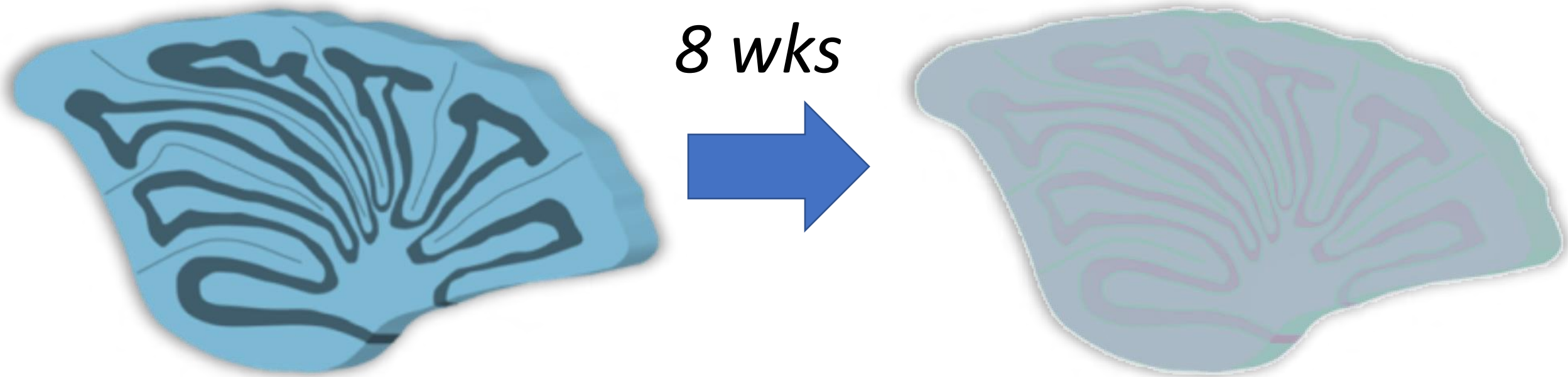
Motility
Central pair microtubules
Radial spokes
Nexin-dynein regulatory complex
Outer and inner dynein arms

Structure and signaling
Axoneme
Ciliary membrane
Signalling complexes

Assembly and compartmentalization
Centriole
Distal appendages
Subdistal appendages
Transition zone
Centriolar satellites

Validation in organotypic slice cultures (COCS)

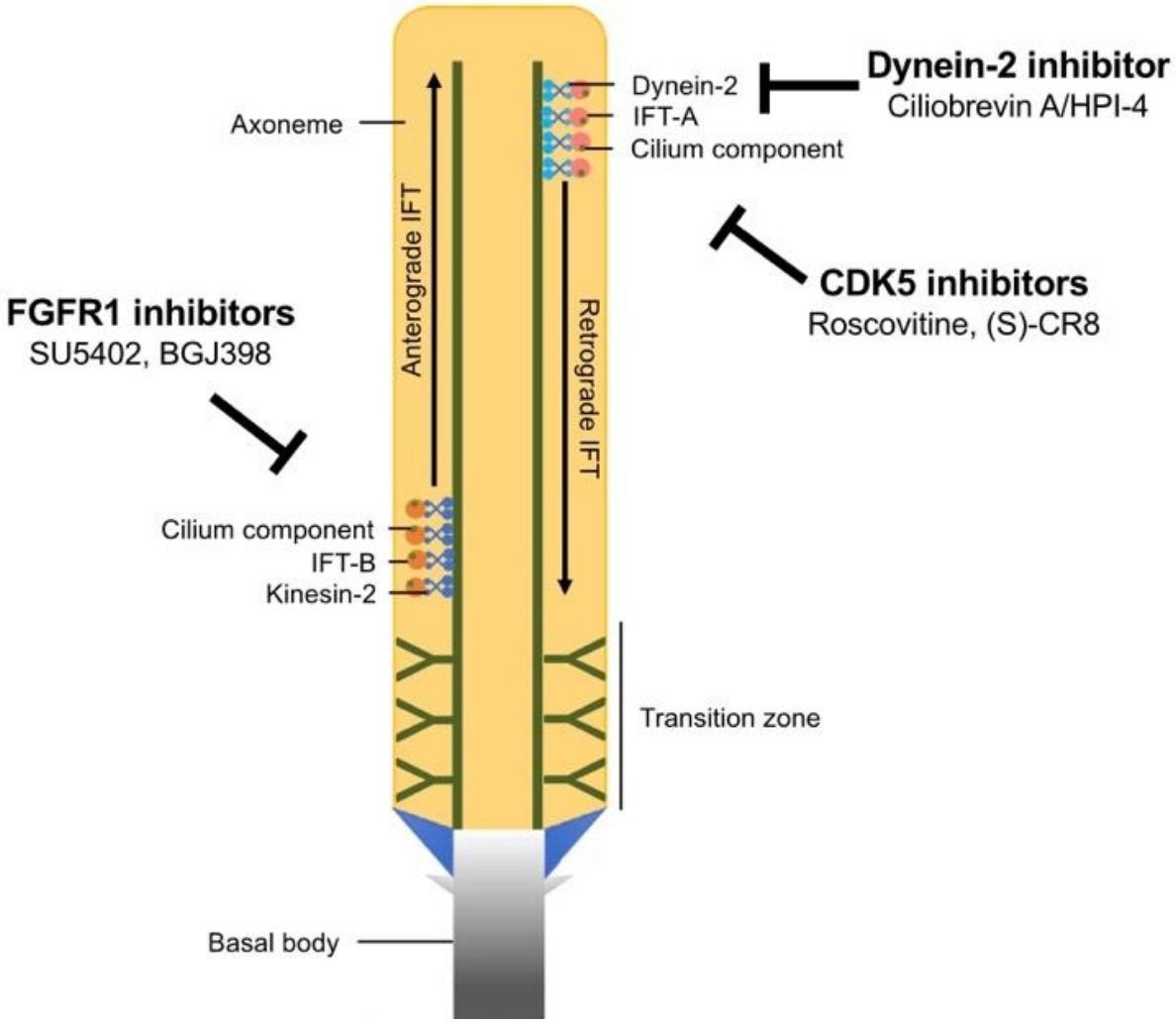
Prion infection
8 wks

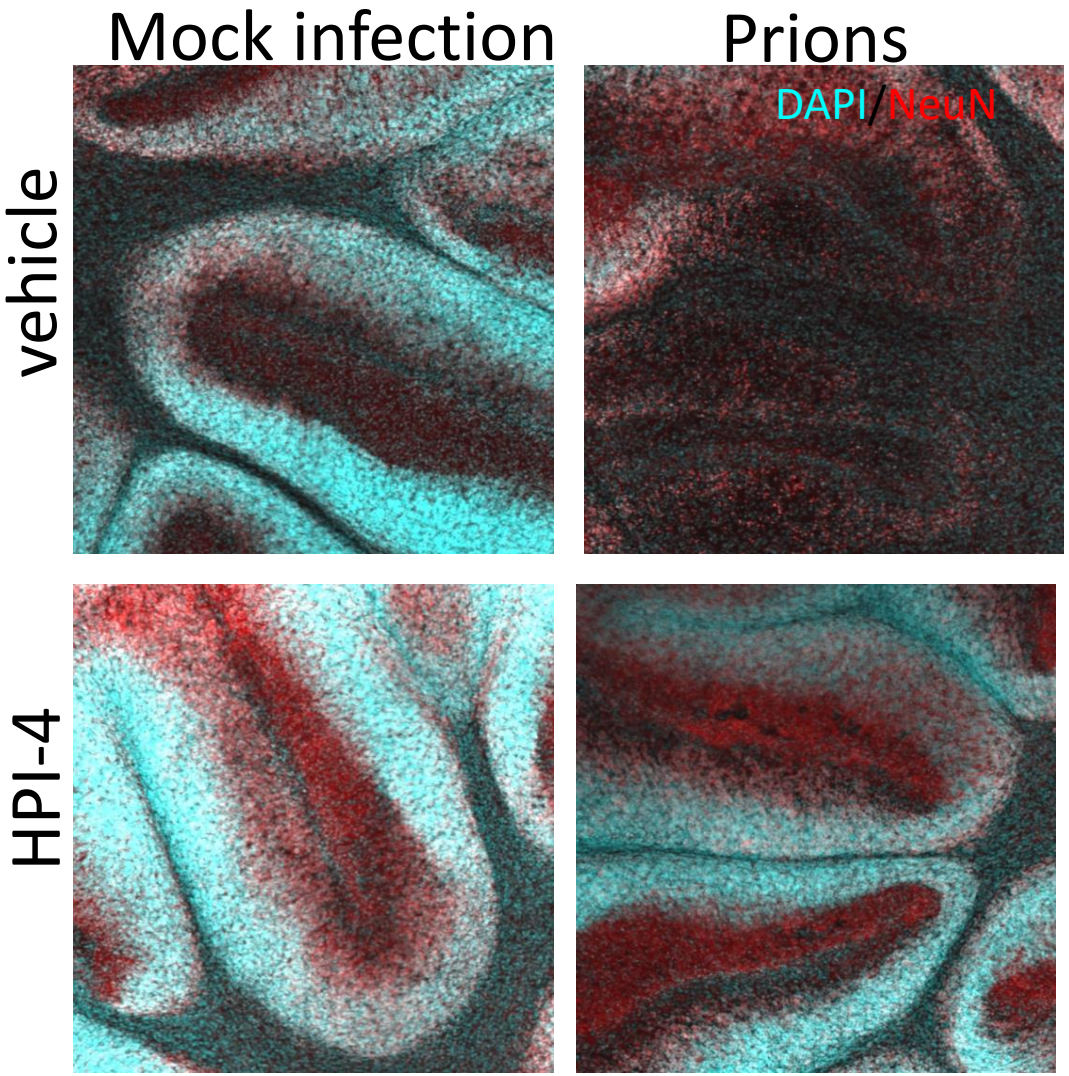


Normal morphology

Cerebellar granule cell degeneration

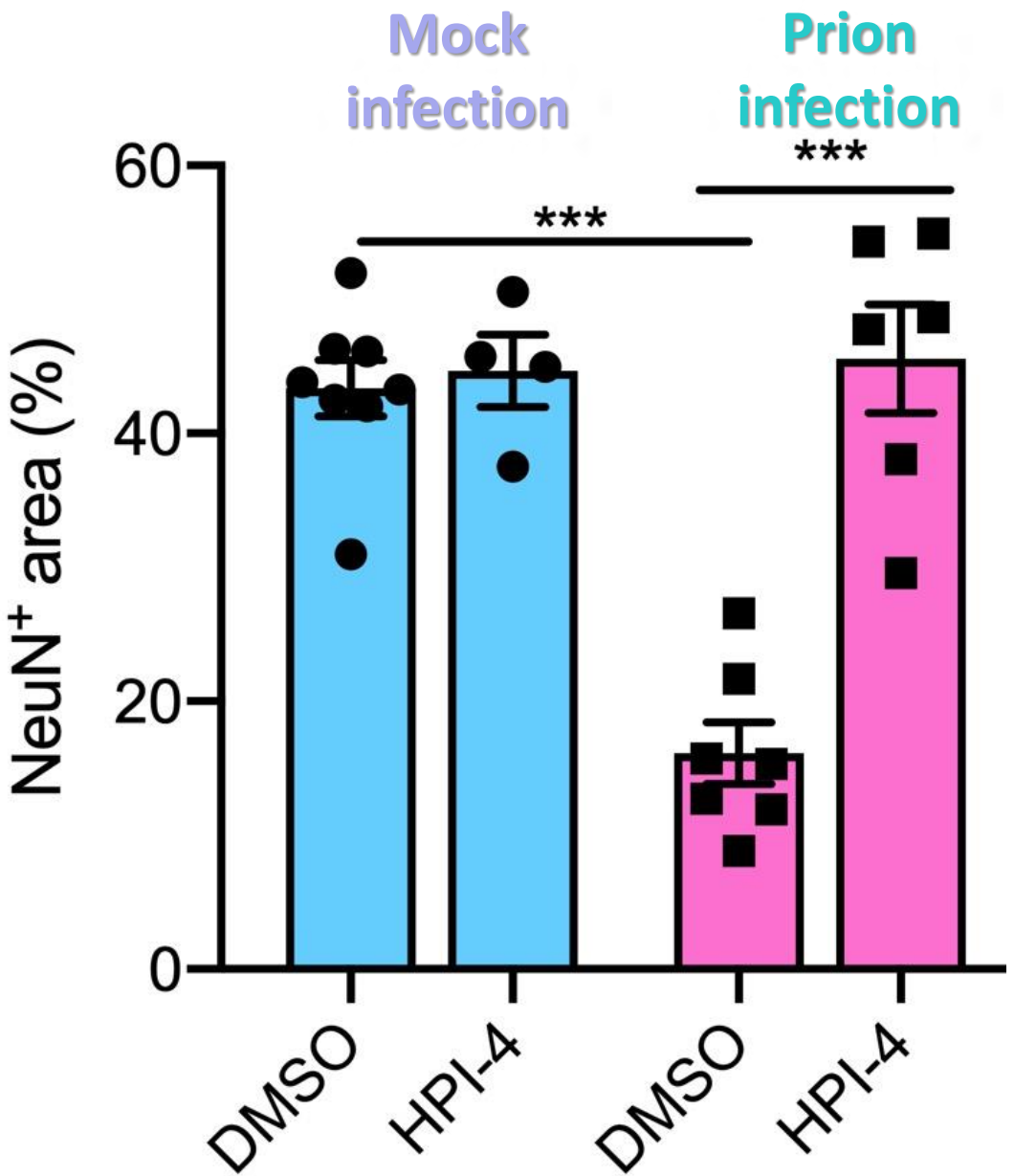
Inhibition of ciliogenesis rescues prion-induced neurodegeneration *ex vivo*



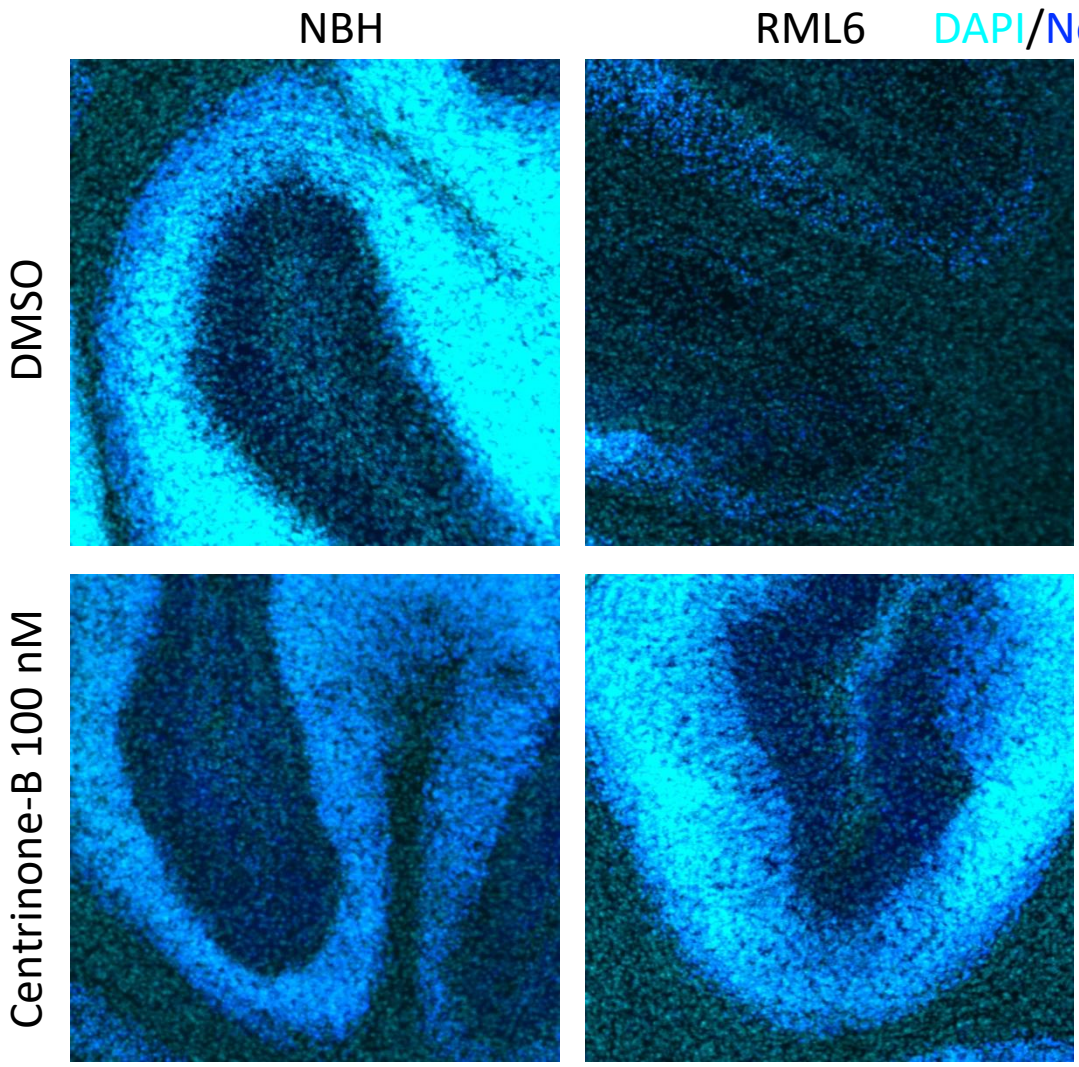


HPI-4: Hedgehog antagonist inhibits ciliogenesis

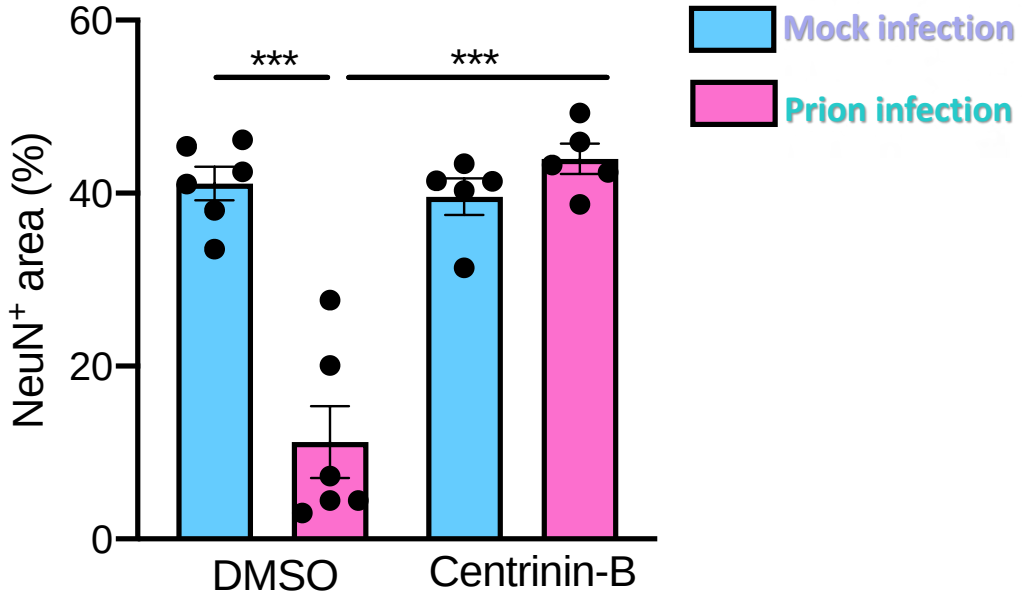
Inhibition of ciliogenesis rescues prion-induced neurodegeneration *ex vivo*



Inhibition of ciliogenesis with Centrinone-B blocks prion toxicity

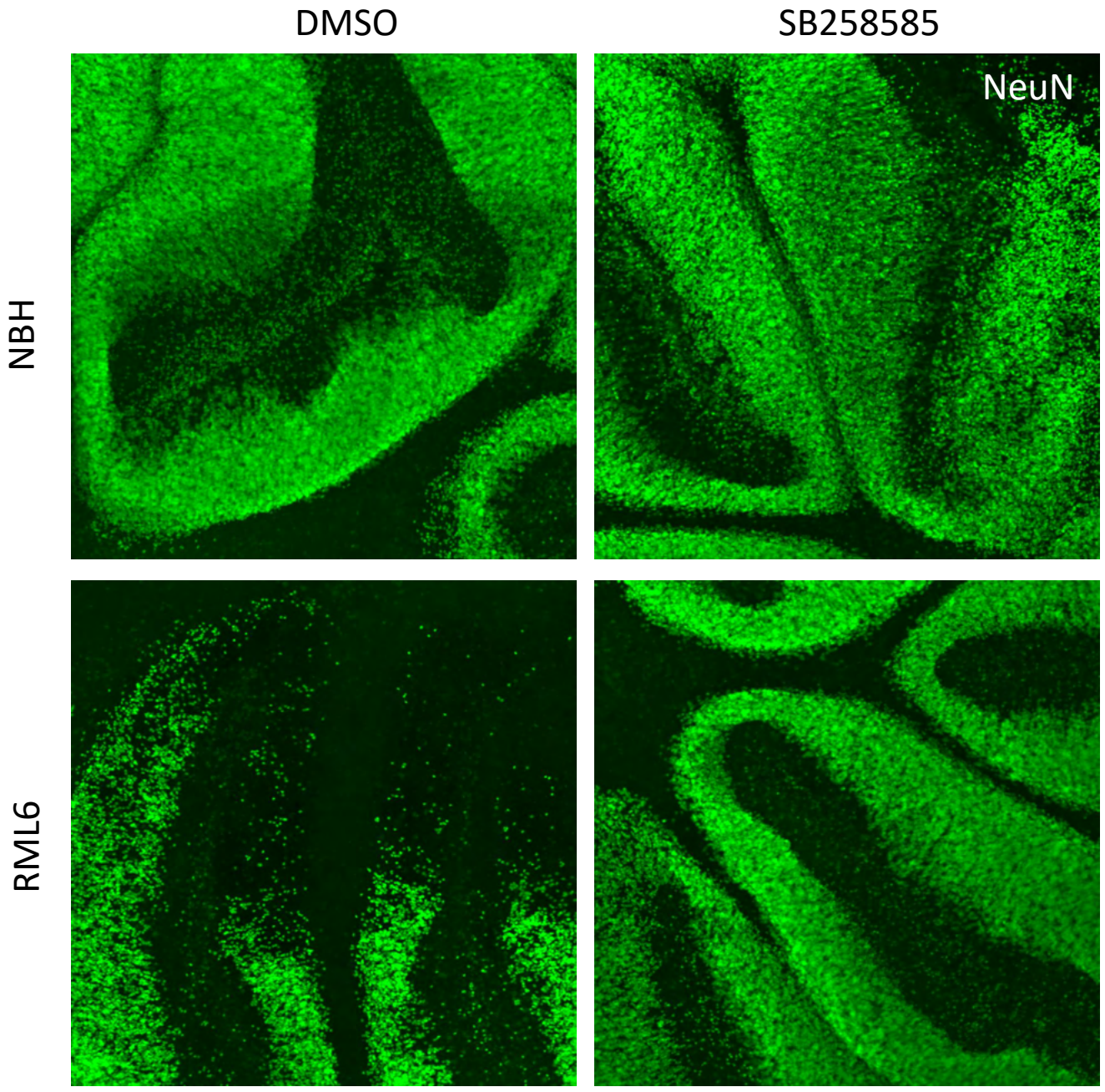


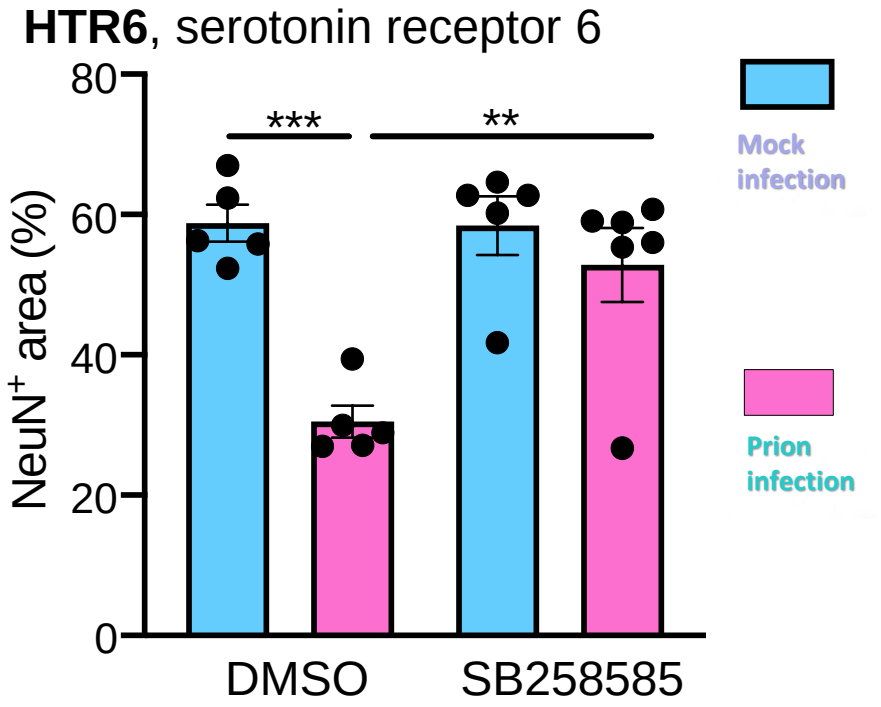
COCS at 55 days post culturing



Centrinone-B:
PLK4 inhibitor

Inhibition of HTR6 alleviates prion toxicity

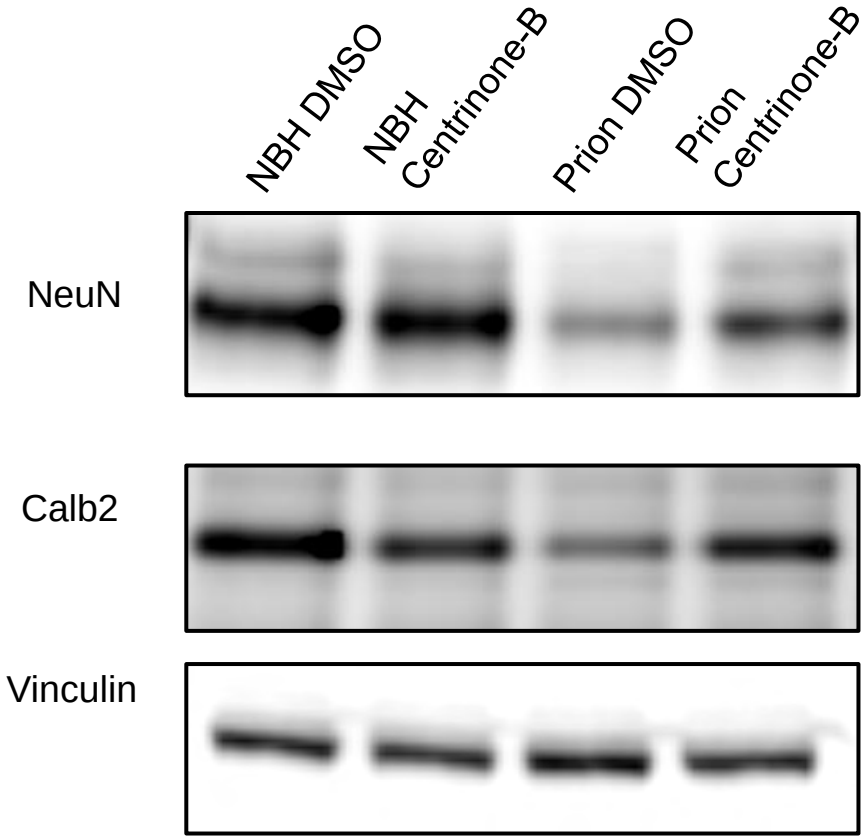


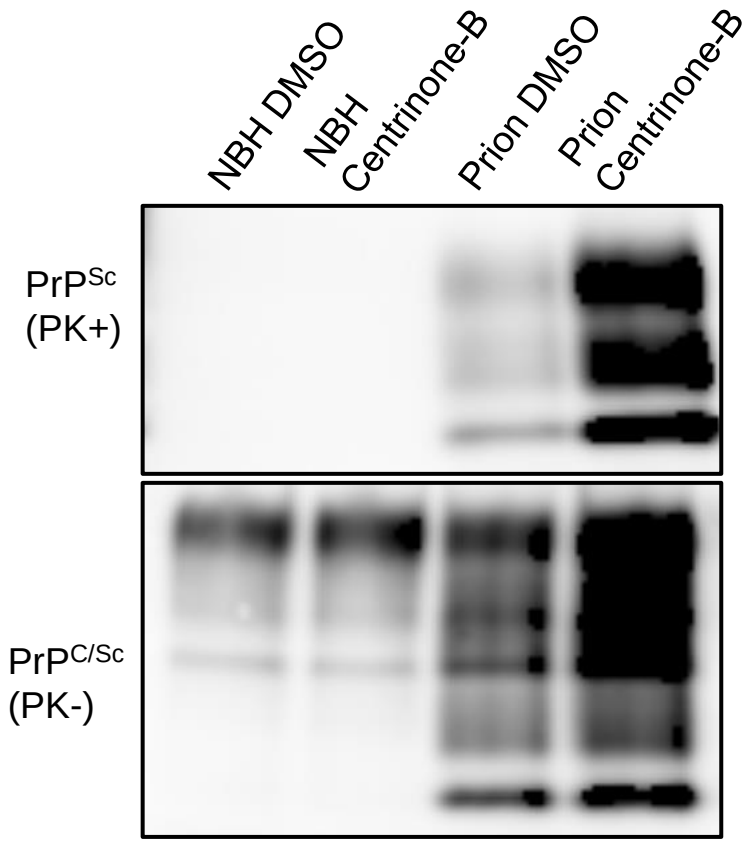


Cultured organotypic cerebellum slice (COCS)

51 days post culturing;
SB258585 : HTR6 specific inhibitor.

Inhibition of ciliogenesis targets prion-induced toxicity but does not reduce PK-resistant prion protein *in sectione*





Centrinone-B: ciliogenesis inhibitor targeting PLK4;
Calb2: Granular cell marker;
Cerebellum slices (Tga20, Prnp over-expressed strain) at 55 days post culturing

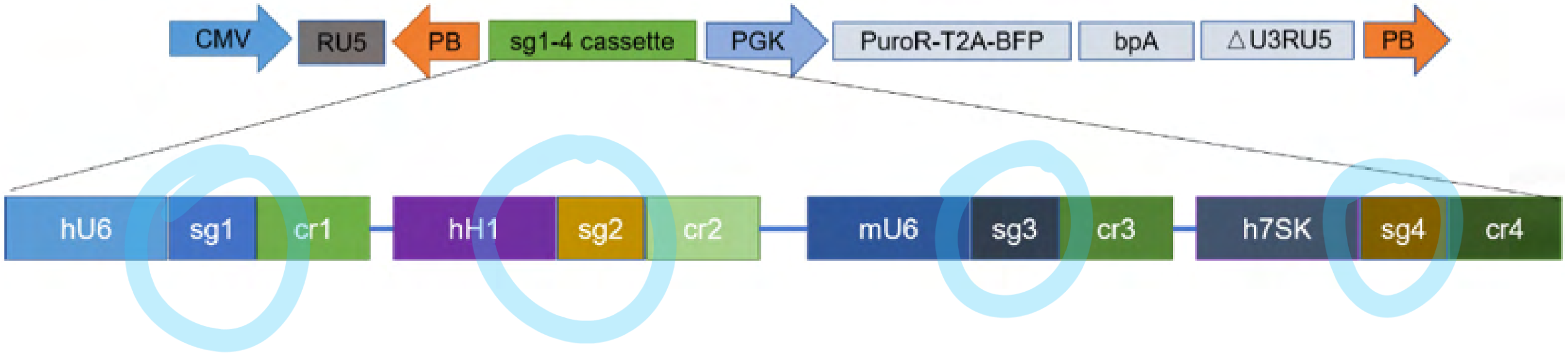
Next-generation arrayed libraries - why???

1. Pooled libraries are good for lethality screening, but inadequate for **biochemical, morphological** and **non-autonomous phenotypes**.

2. Single sgRNA efficacy is low, variable and unpredictable.

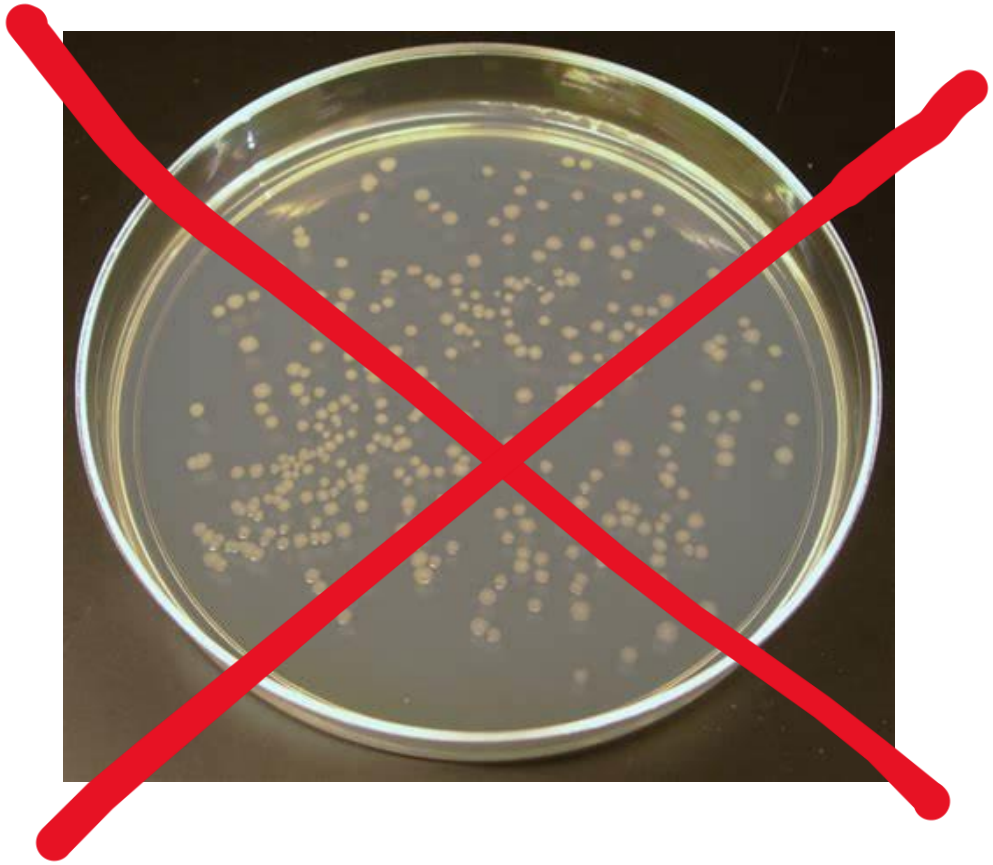
3. Most libraries disregard DNA polymorphisms among humans.

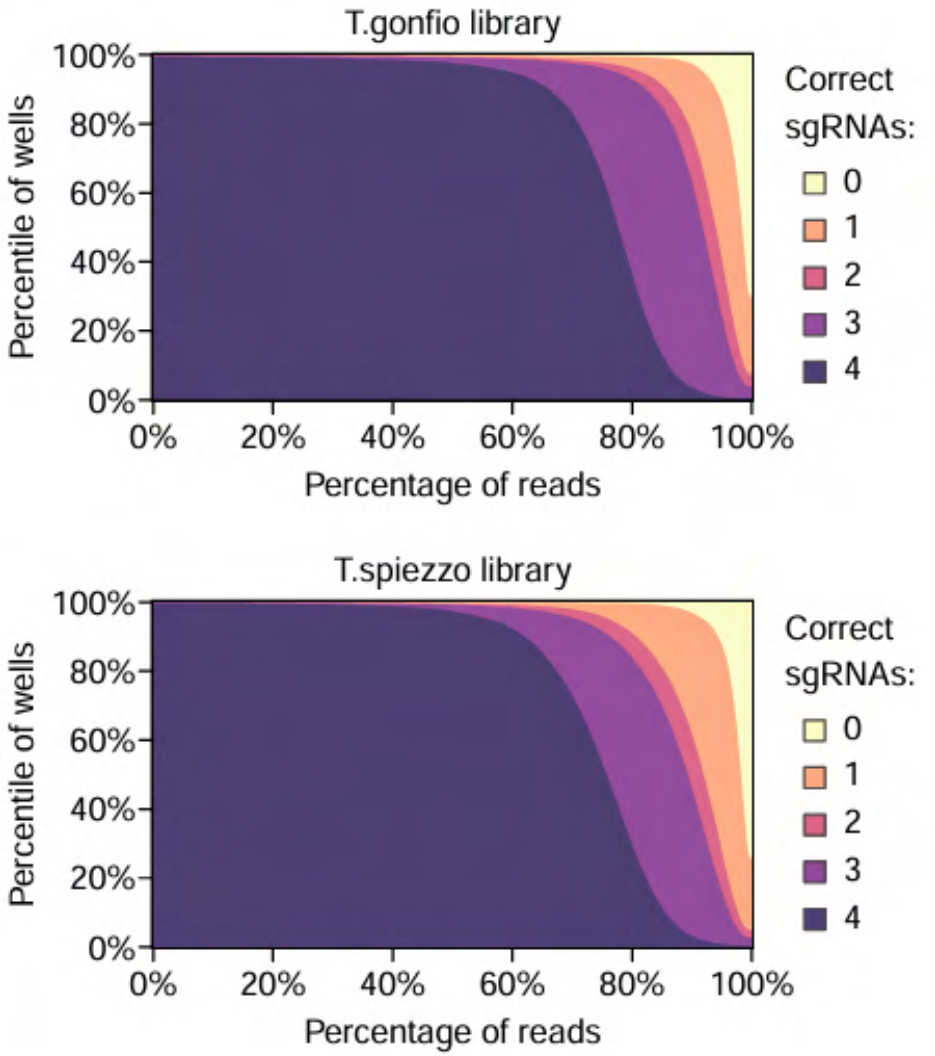
Arrayed libraries for indexed genome-wide CRISPRcut, CRISPRact and CRISPRoff screens



- 4 sgRNA/plasmid
- Lentiviral backbone
- PiggyBac transposon elements
- Puromycin and blue-fluorescent protein markers
- Designed to be tolerant to the most common human genetic polymorphisms.

APPEAL does not require agarose colony picking



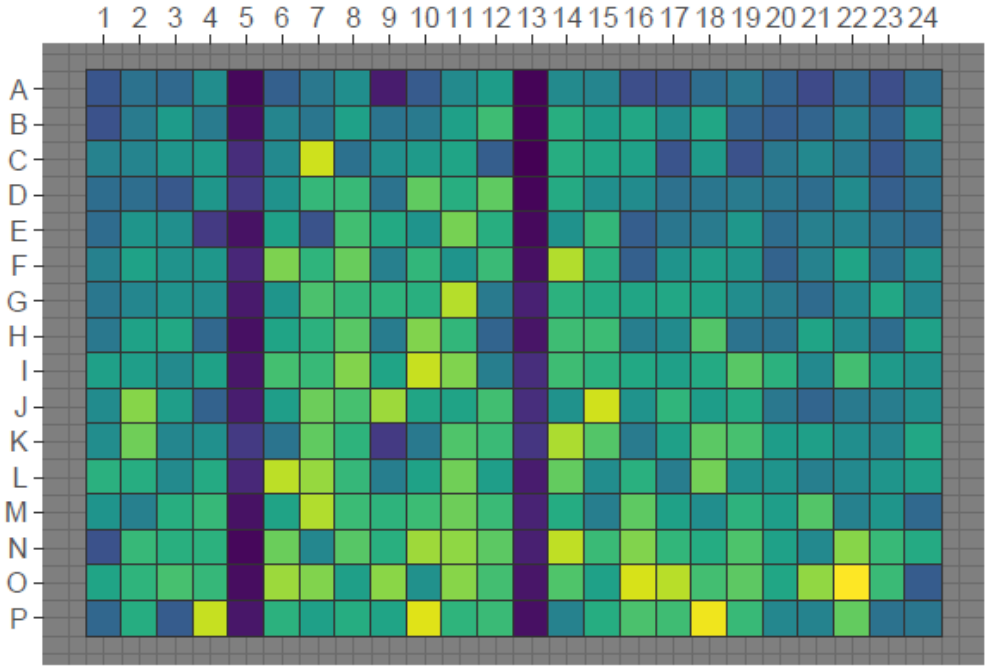


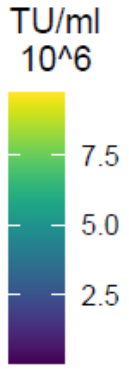
19’820 CRISPRo plasmids (T.spiezzo)

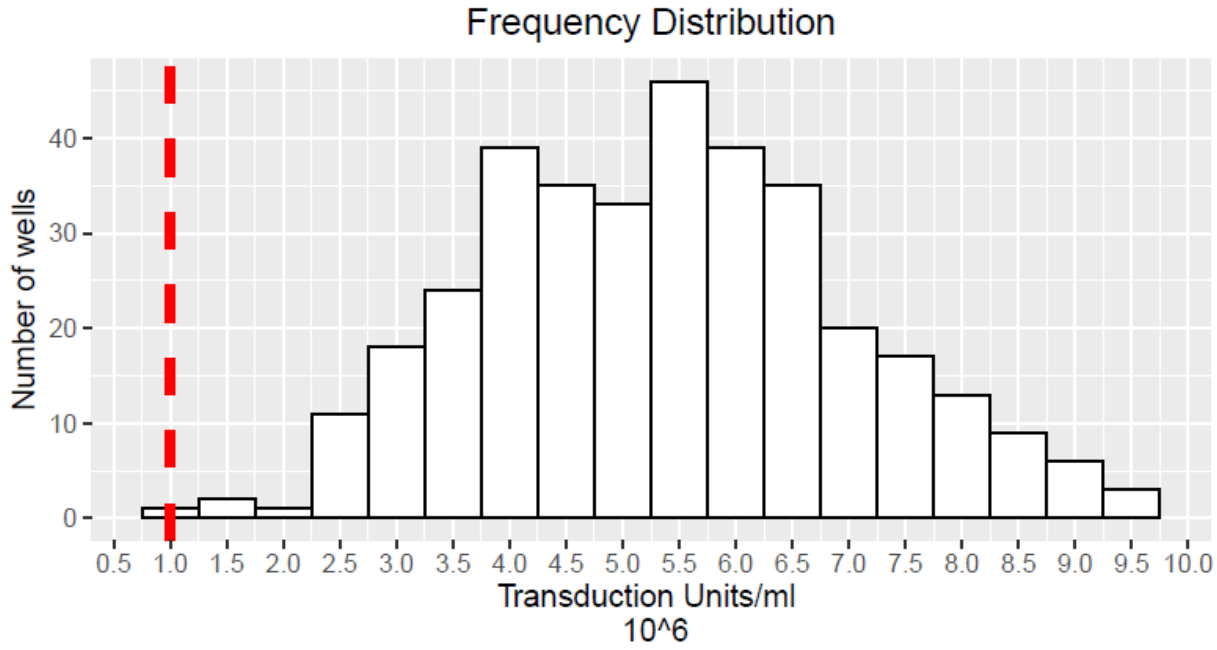
22’326 CRISPRa plasmids (T.gonfio)

Jiang-An Yin et al.
Nature Biomedical Engineering, 2024

High-throughput high-titer lentivirus production

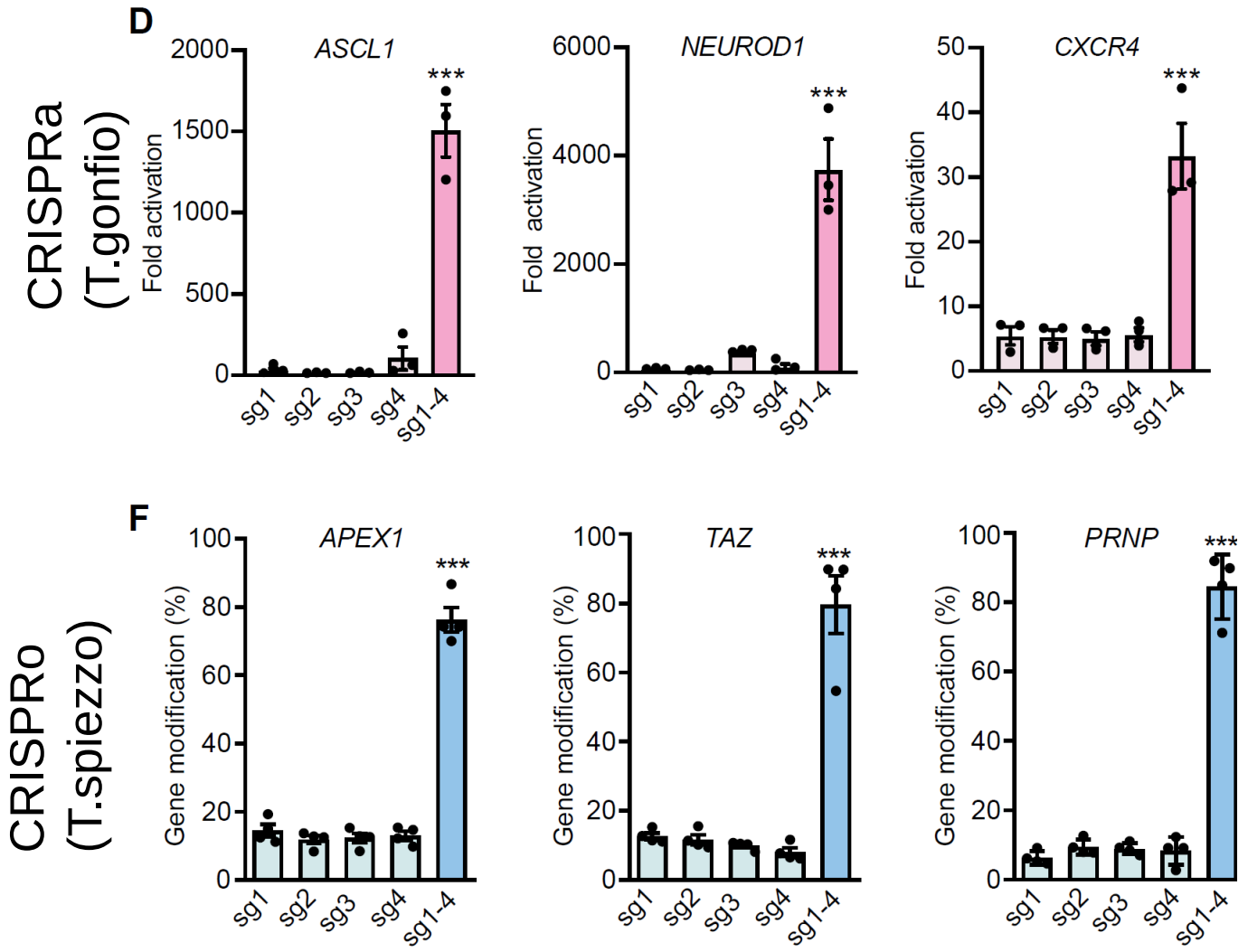






Yin et al, 2024, *Nat. Biomed. Eng.*

4sgRNAs are better than 1 sgRNA



Glucocerebrosidase and Parkinson's disease

GBA mutations are the most common genetic risk factor for PD development (independent of GD)

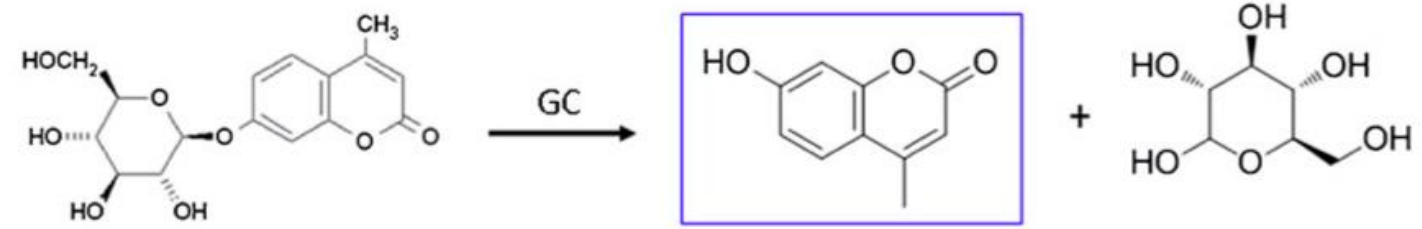
Incomplete penetrance: not all *GBA* mutation carriers develop PD (~10-30% by age 70)

→ Genetic modifiers most likely contribute to disease development and phenotype in GD and PD!

Kathi Ging, Jiang-An Yin et al.
npj Parkinsons Dis. **10**, 192 (2024)
https://doi.org/10.1038/s41531-024-00819-7

A genome-wide arrayed activation screen for GBA modifiers

Ex=365nm and Em=440nm



4-Methylumbelliferyl β-D-glucopyranoside

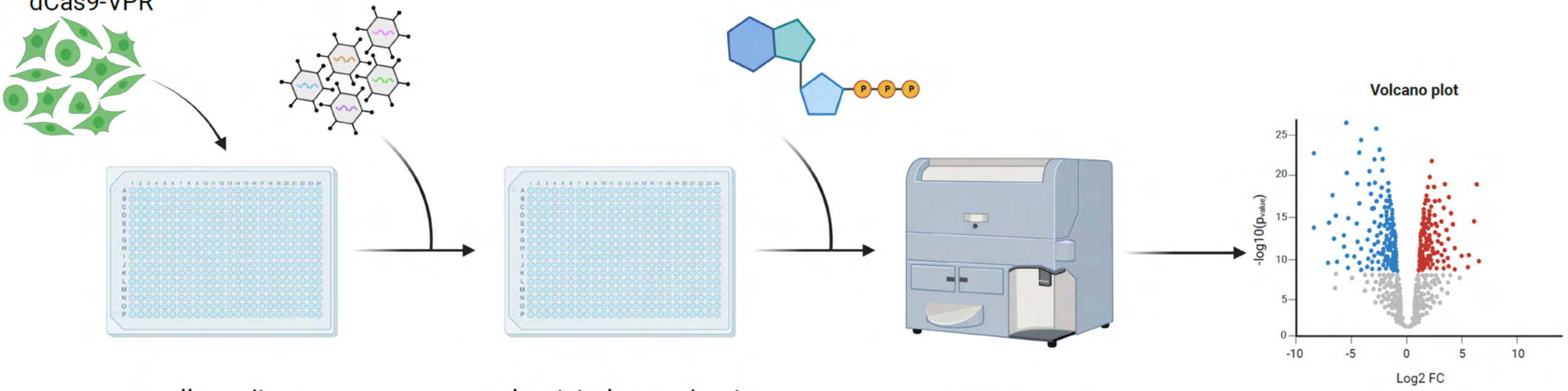
4-Methylumbelliferone

Glucose

LN229
GBA-L444P;
dCas9-VPR

T.gonfio library

4MU-Glc



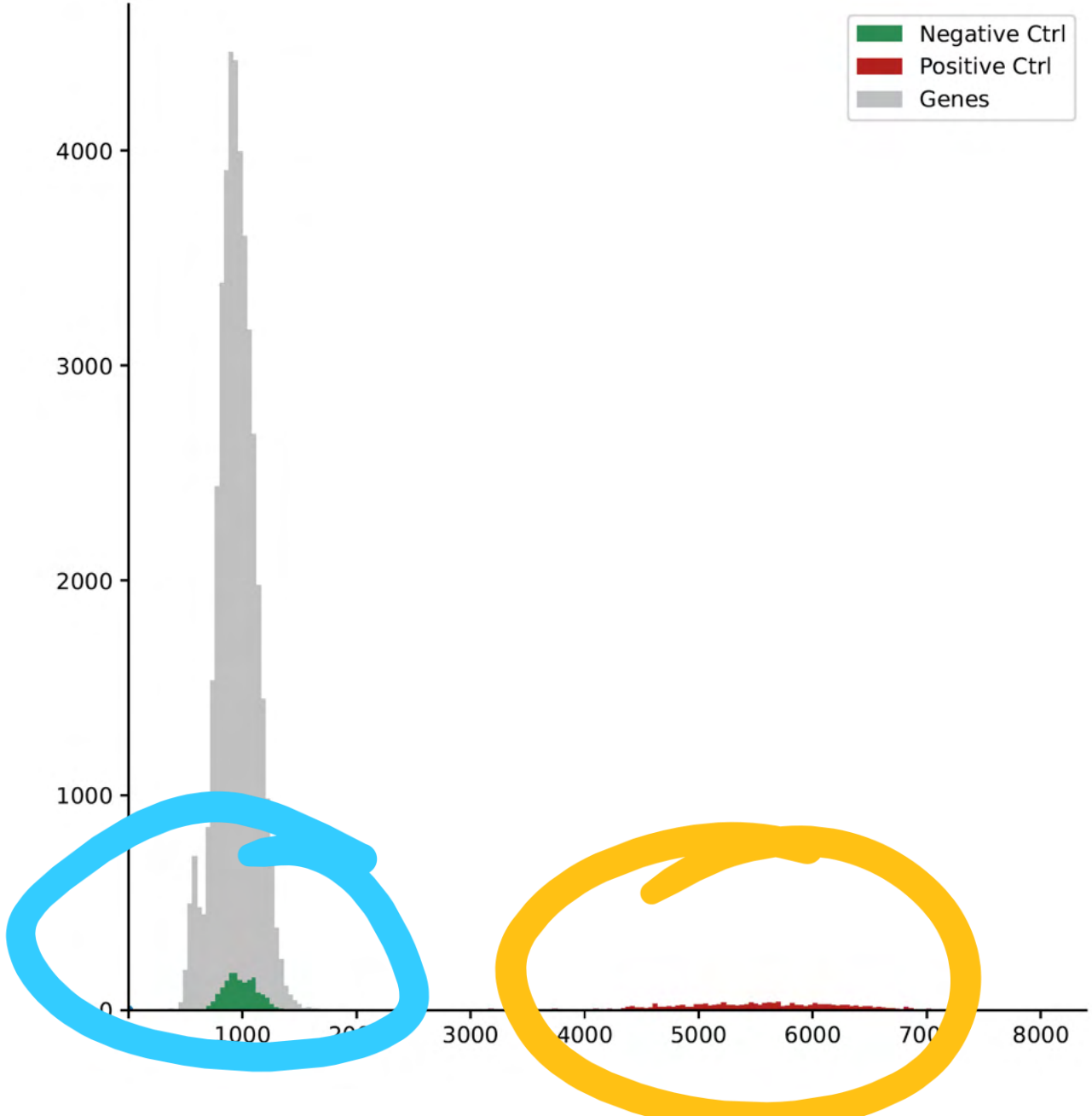
cell seeding

lentiviral transduction and incubation 5 days

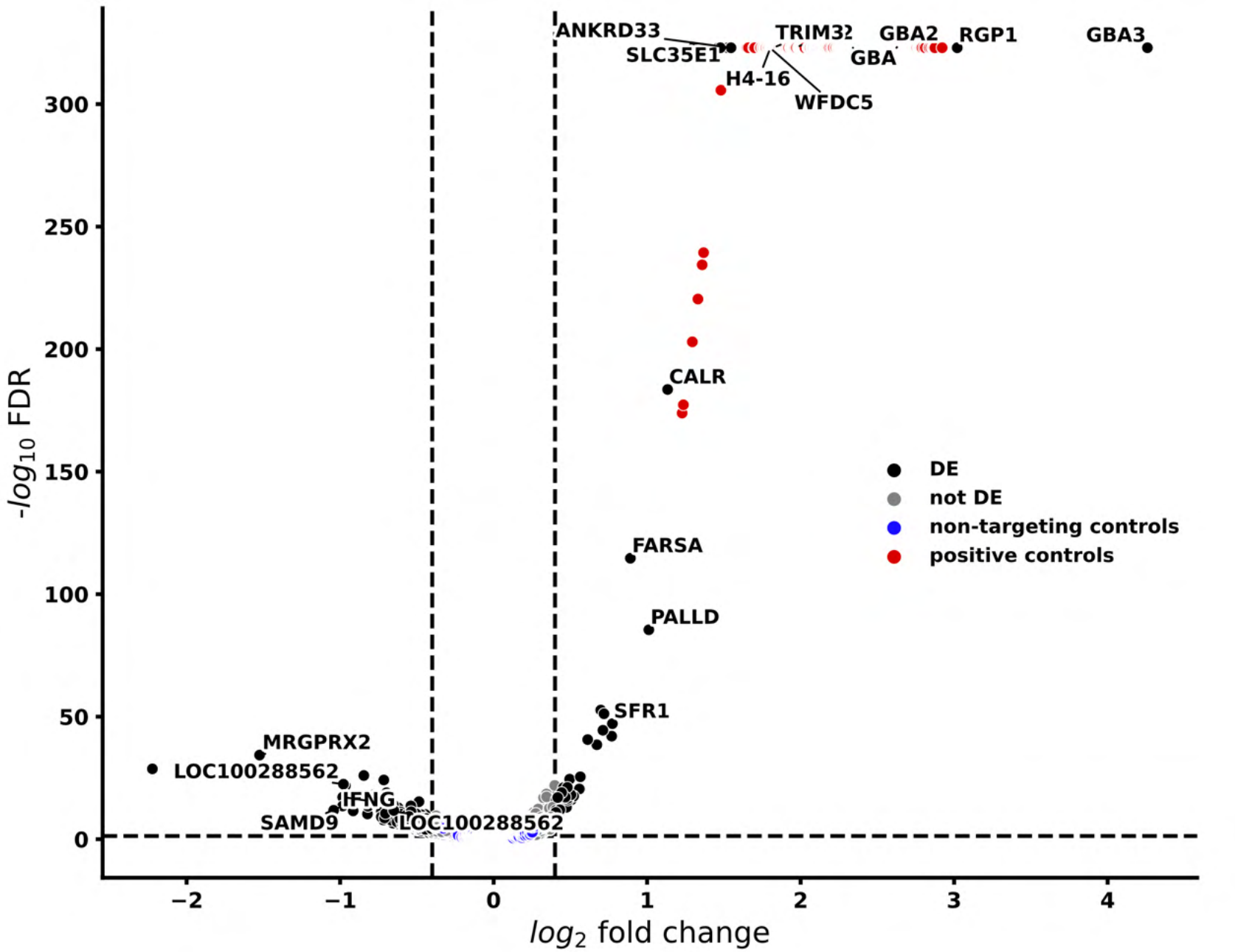
plate reading

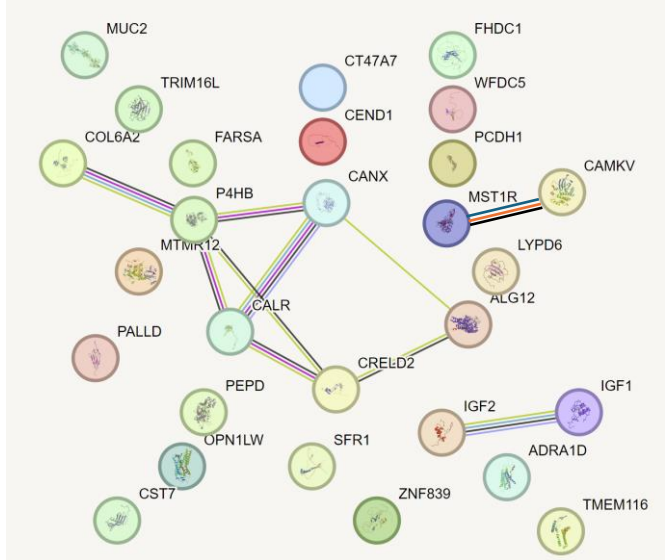
data analysis

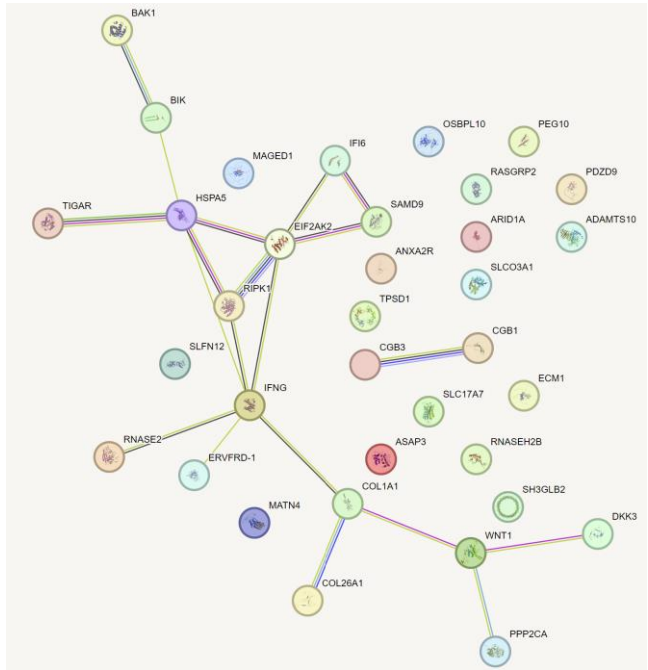
Excellent separation between negative and positive controls



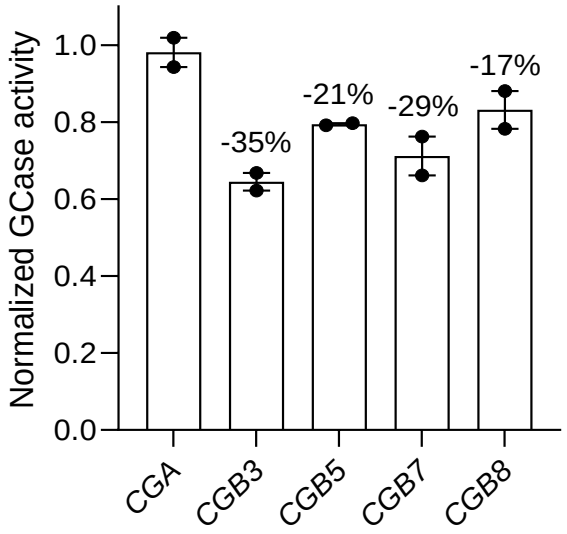
Genome wide modifiers of GCase







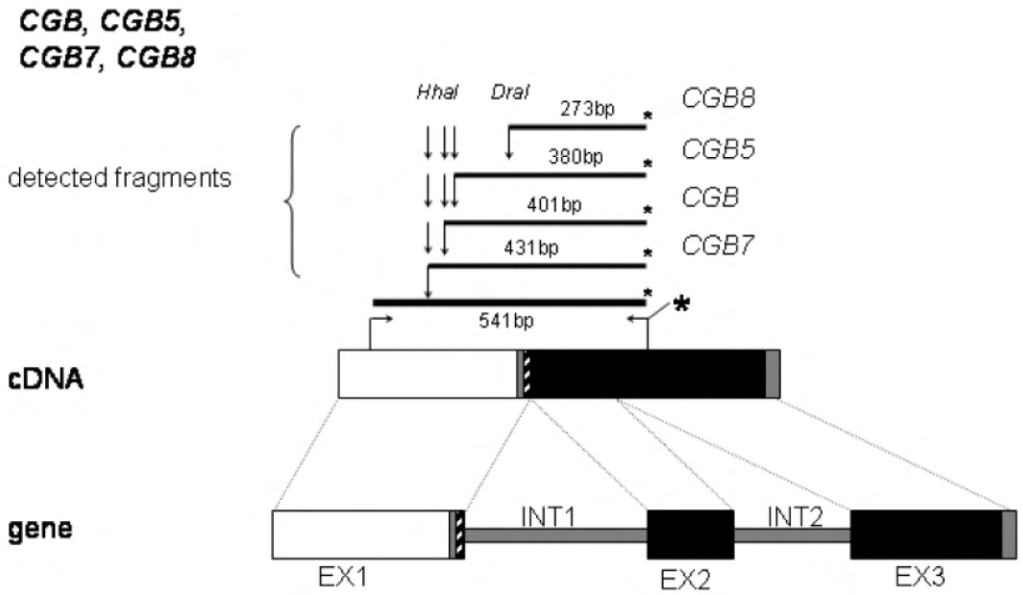
Human chorionic gonadotropin (hCG) and PD



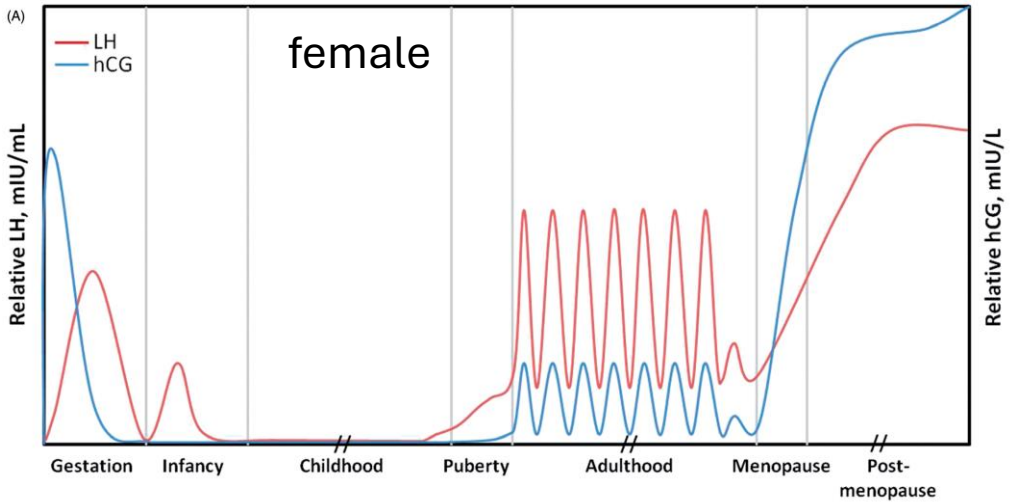
Parkinson's Disease in Pregnancy: A Case Report and Review of the Literature

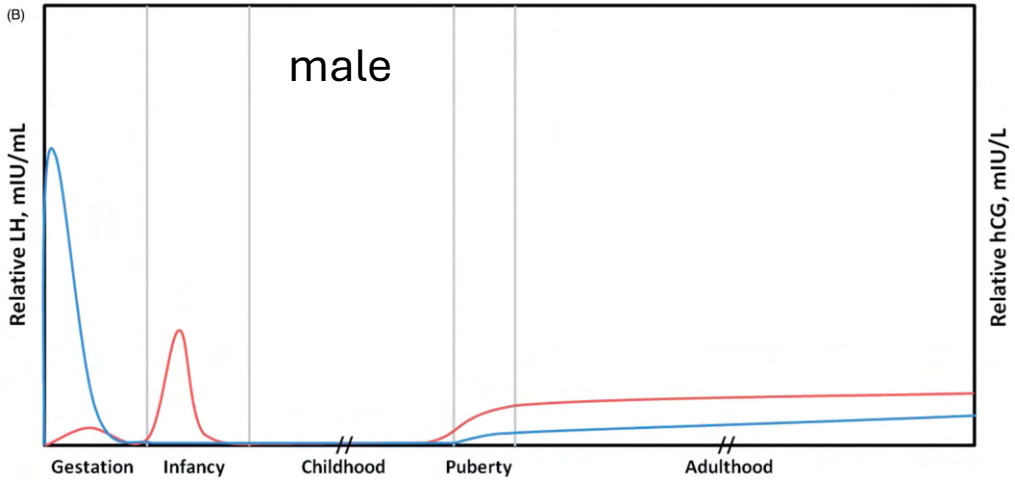
Sara Olivola[1], Serena Xodo[1], Enrica Olivola[2], Fabiana Cecchini[1], Ambrogio Pietro Londero[1] and Lorenza Driul[1]*

[1] Department of Gynaecology and Obstetrics, School of Medicine of Udine, Udine, Italy, [2] IRCCS Istituto Neurologico Mediterraneo (INM) Neuromed, Pozzilli, Italy



Kristiina Rull and Maris Laan, 2005

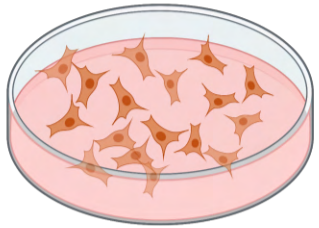




Janet Choi and Johan Smitz, 2014

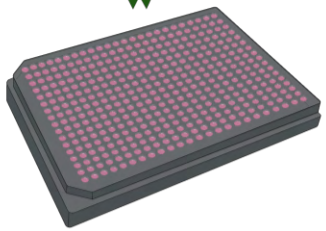
Jiang-An Yin et al unpublished

Optical CRISPR screen for modifiers of α-Synuclein aggregation

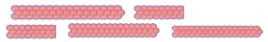


dCas9 and α-Syn
Overexpressing cells

Transfection with
CRISPRa plasmids

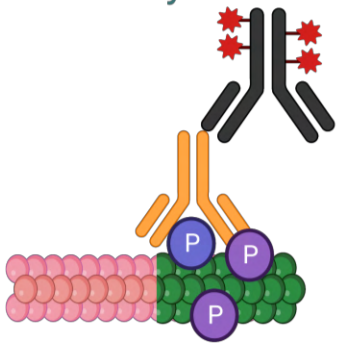


An arrayed screen in
384-well plate

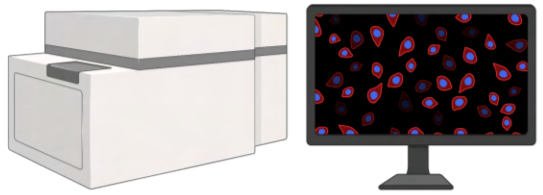


α-Synuclein Preformed Fibrils
Treatment

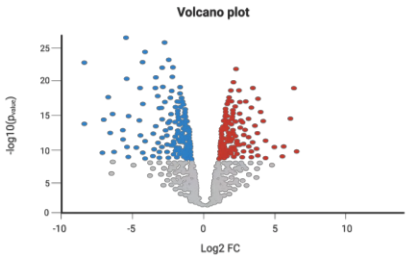
P-syn/81A Antibody



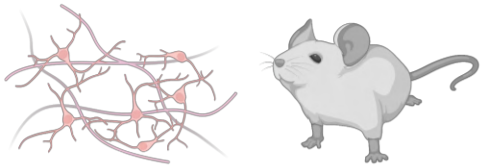
Immunostaining with
anti-pSer129 Antibody



High-throughput Imaging and
Image Analysis



Data analysis and Hits Selection

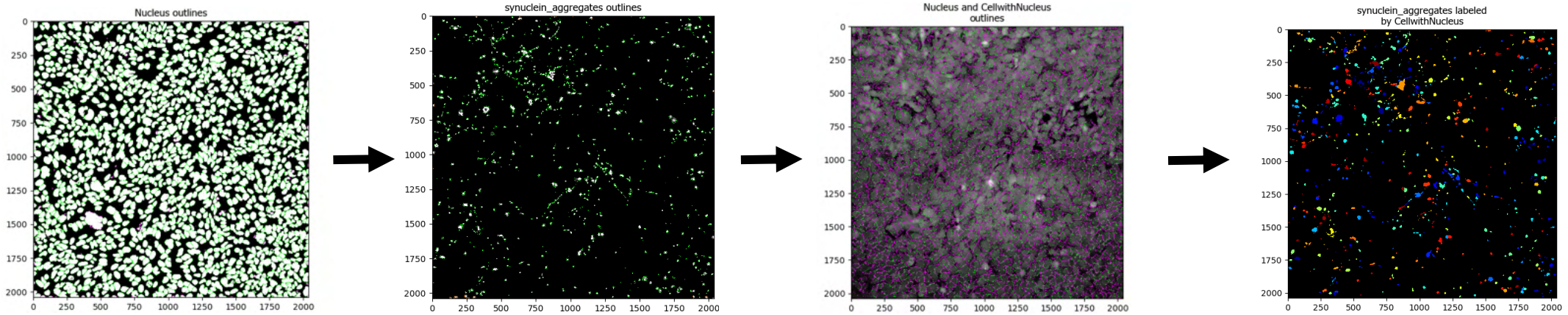


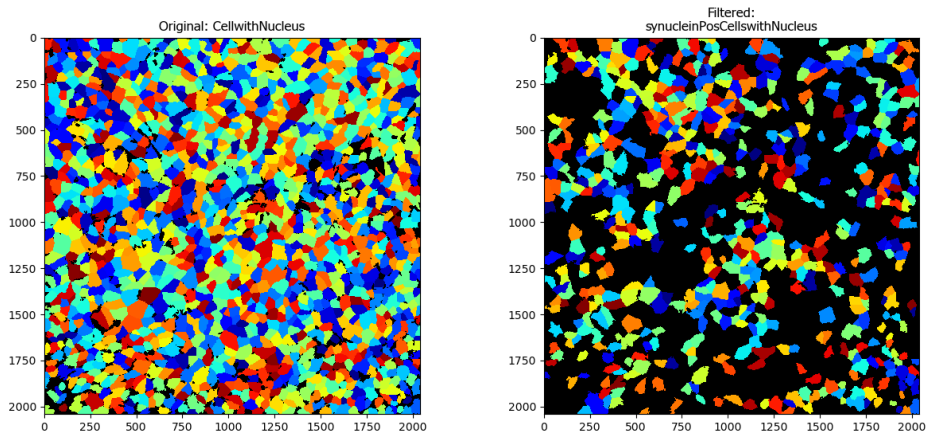
Hit validation in neuronal and
animal models

Sandesh Neupane, Dalila Vena, Elena De Cecco

Image Analysis Pipeline

Identification and quantification of cells positive for a-synuclein aggregates





1. Pixel classification and objects segmentation (nuclei and fibrils)
2. Secondary objects segmentation (cells)
3. Quantification of total cells
4. Quantification of cells that contain fibrils

Athena Economides

HCS-AggreQuant/README.md at

https://github.co...

173 lines (122 loc) · 5.85 KB

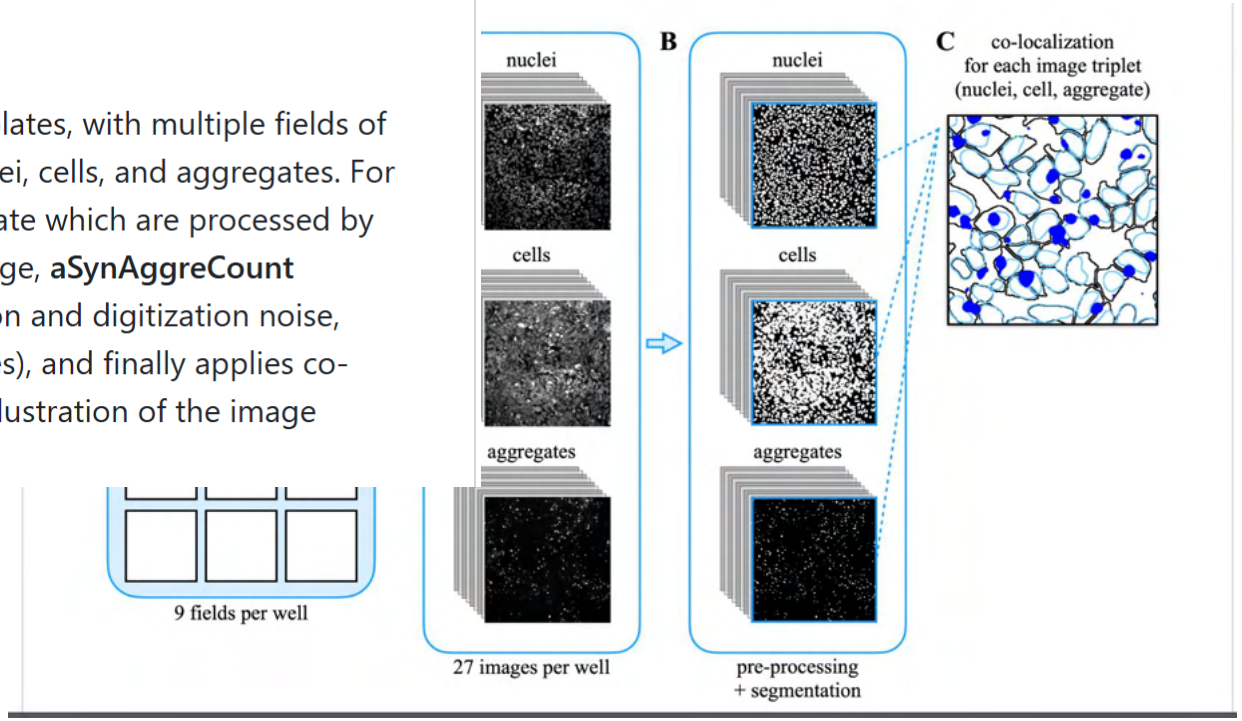
Preview | Code | Blame

Raw

aSynAggreCount

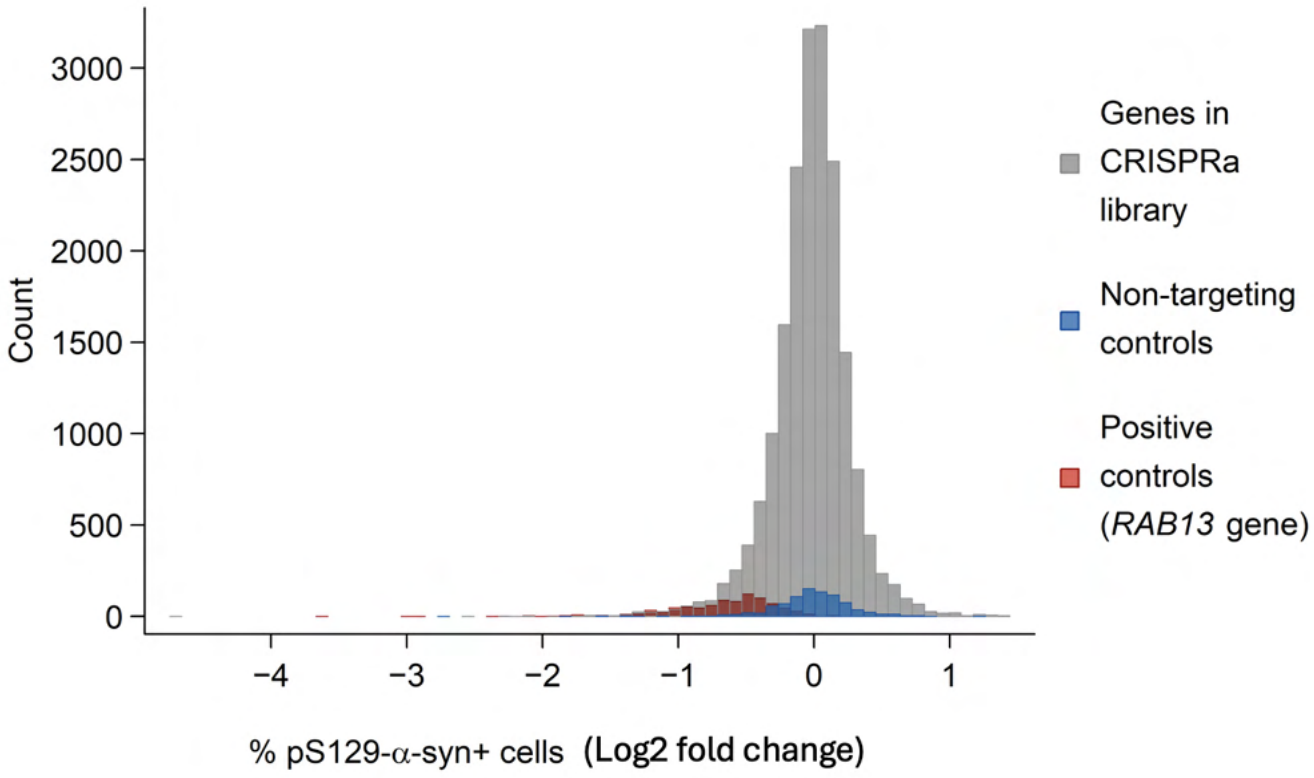
A codebase for automated analysis of High Content Screens.

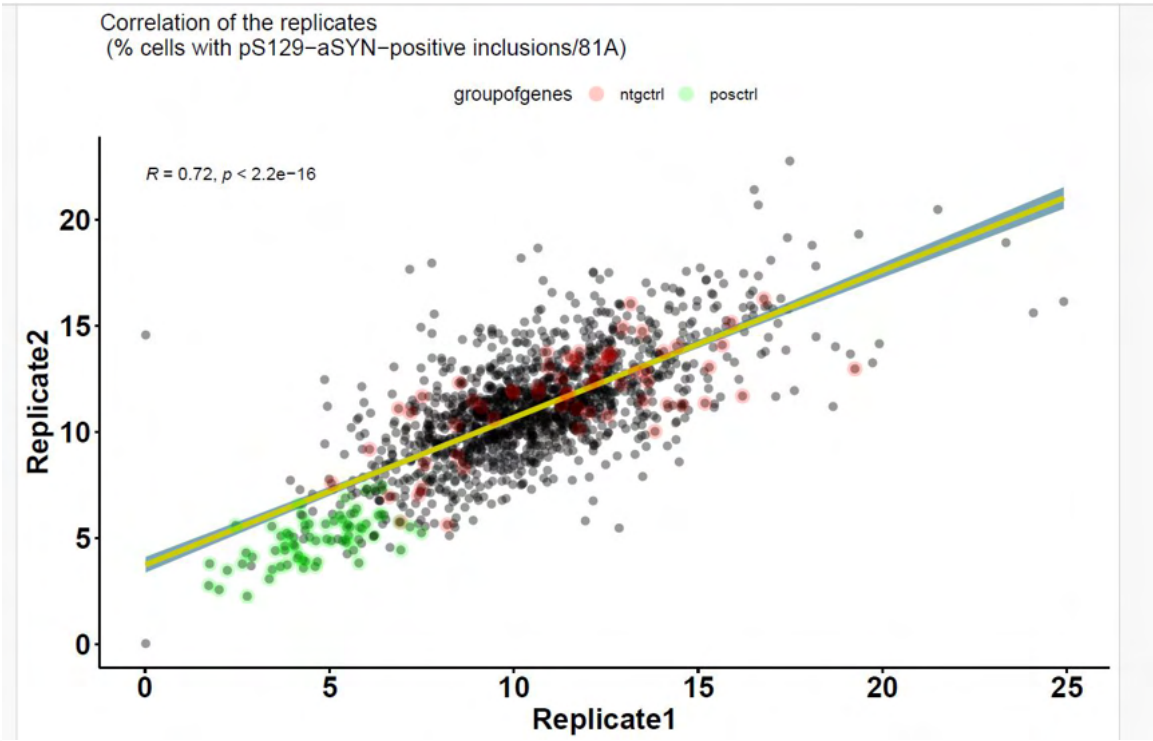
The input image-data are assumed to be generated from High Content Screen plates, with multiple fields of view acquired per well, and 3 channels recorded per field, corresponding to nuclei, cells, and aggregates. For a 384-well plate with 9 fields per well, 10'368 images are acquired in total per plate which are processed by the **aSynAggreCount** package to quantify aggregate-positive cells. For each image, **aSynAggreCount** performs image pre-processing to correct for the presence of uneven illumination and digitization noise, then performs segmentation of the structures of interest (nuclei, cells, aggregates), and finally applies co-localization analysis to characterize the presence of aggregates inside cells. An illustration of the image processing pipeline is shown below.



Athena Economidou
https://github.com/aecon/AggreQuant

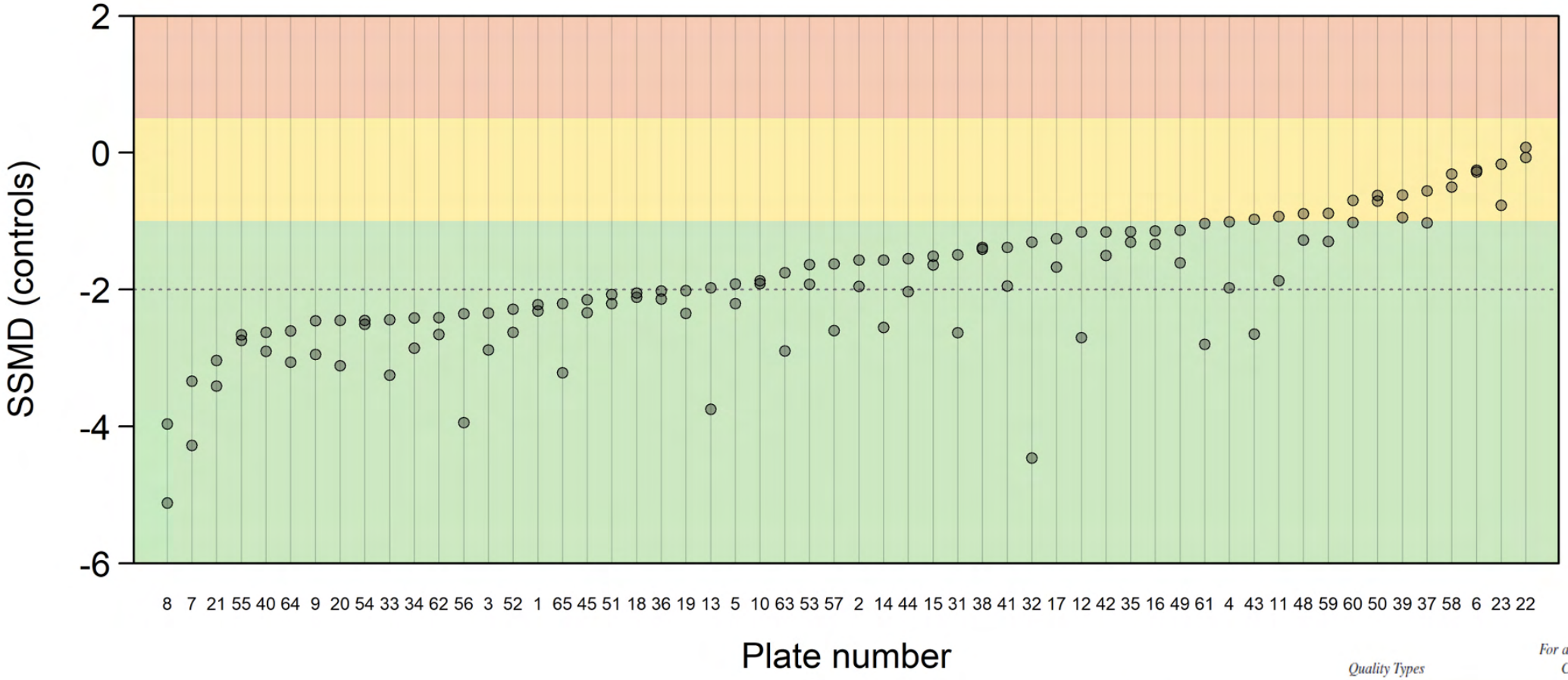
Genome-wide screen





Dalila Vena, Elena De Cecco

Quality control: SSMD score



$$\mathrm{SSMD} = \frac{\bar{X}_P - \bar{X}_N}{\sqrt{s_P^2 + s_N^2}}.$$

Quality Types	*For a Moderate Control*
Excellent	$\hat{\beta} \le -2$
Good	$-2 < \hat{\beta} \le -1$
Inferior	$-1 < \hat{\beta} \le -0.5$
Poor	$\hat{\beta} > -0.5$

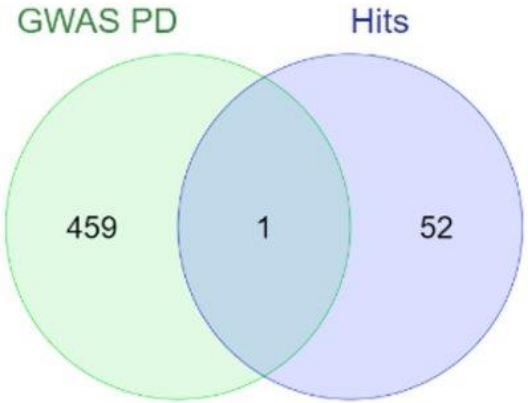
UPREGULATORS

CDH1	Cadherin 1
C18orf54	Chromosome 18 Open Reading Frame 54
TMEM101	Transmembrane Protein 101
WDR66	Cilia And Flagella Associated Protein 2
FOXA3	Forkhead Box A3
DEFB105A	Defensin Beta 105A
TRIM49	Tripartite Motif Containing 49
ADPRHL1	ADP-Ribosylhydrolase Like 1
ADRB1	Adrenoceptor Beta 1
CHRM3	Cholinergic Receptor Muscarinic 3
PIM3	Pim-3 Proto-Oncogene, Serine/Threonine Kinase
RNF227	Ring Finger Protein 227
HAPLN4	Hyaluronan And Proteoglycan Link Protein 4
BCL2L13	BCL2 Like 13
ANKRD61	Ankyrin Repeat Domain 61
BCL2L2-PABPN1	BCL2L2-PABPN1 Readthrough
AGT	Angiotensinogen
ZDHHC3	Zinc Finger DHHC-Type Palmitoyltransferase 3
TNFRSF19	TNF Receptor Superfamily Member 19
LRRC8C	Leucine Rich Repeat Containing 8 VRAC Subunit C
THNSL2	Threonine Synthase Like 2
HEATR5A	HEAT Repeat Containing 5A
FZD9	Frizzled Class Receptor 9
FAM106B	Family With Sequence Similarity 106 Member B
ITPRID1	ITPR Interacting Domain Containing 1
MYORG	Myogenesis Regulating Glycosidase (Putative)
ERICH2	Glutamate Rich 2
AFF1	ALF Transcription Elongation Factor 1
C17orf100	Chromosome 17 Open Reading Frame 100
ZNF765	Zinc Finger Protein 765
CFAP57	Cilia And Flagella Associated Protein 57

DOWNREGULATORS

FARP1	FERM, ARH/RhoGEF And Pleckstrin Domain Protein 1
CD63	CD63 Molecule
SIK2	Salt Inducible Kinase 2
GPR50	G Protein-Coupled Receptor 50
SRGAP1	SLIT-ROBO Rho GTPase Activating Protein 1
CA10	Carbonic Anhydrase 10
SPDYE5	Speedy/RINGO Cell Cycle Regulator Family Member E5
MAGED2	MAGE Family Member D2
VCAN	Versican
PSG6	Pregnancy Specific Beta-1-Glycoprotein 6
PPFIA2	PTPRF Interacting Protein Alpha 2
LOC101929627	Putative Exonuclease GOR
ARHGAP27	Rho GTPase Activating Protein 27
UNC119B	Unc-119 Lipid Binding Chaperone B
SARS2	Seryl-TRNA Synthetase 2, Mitochondrial
KLHL32	Kelch Like Family Member 32
PIK3R3	Phosphoinositide-3-Kinase Regulatory Subunit 3
COL6A2	Collagen Type VI Alpha 2 Chain
SLC30A2	Solute Carrier Family 30 Member 2
CCT4	Chaperonin Containing TCP1 Subunit 4
TIFAB	TIFA Inhibitor
EEF1AKMT1	EEF1A Lysine Methyltransferase 1
EIF2AK1	Eukaryotic Translation Initiation Factor 2 Alpha Kinase 1

GWAS



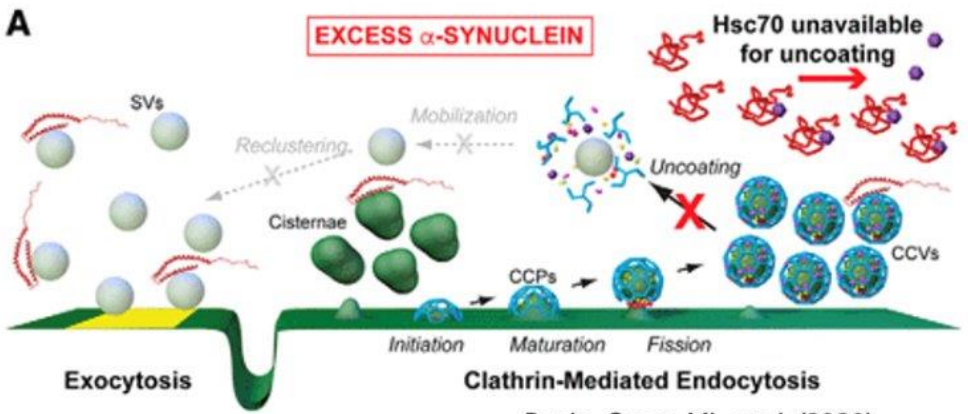
GWAS PD (collection from several association studies MONDO_0005180)

ARHGAP27
Rho GTPase Activating Protein 27

"The encoded protein may play a role in clathrin-mediated endocytosis."
"Increased expression of ARHGAP27 in the brain cortex was associated with decreased risk of PD"

SCREEN	**↑ ARHGAP27 ↓ phospho-α-synuclein**
GWAS	**↑ ARHGAP27 ↓ PD risk**
LITERATURE	**↑ α-synuclein ↓ clathrin-mediated endocytosis**

"Acute introduction of α-synuclein impairs clathrin-mediated synaptic vesicle endocytosis"

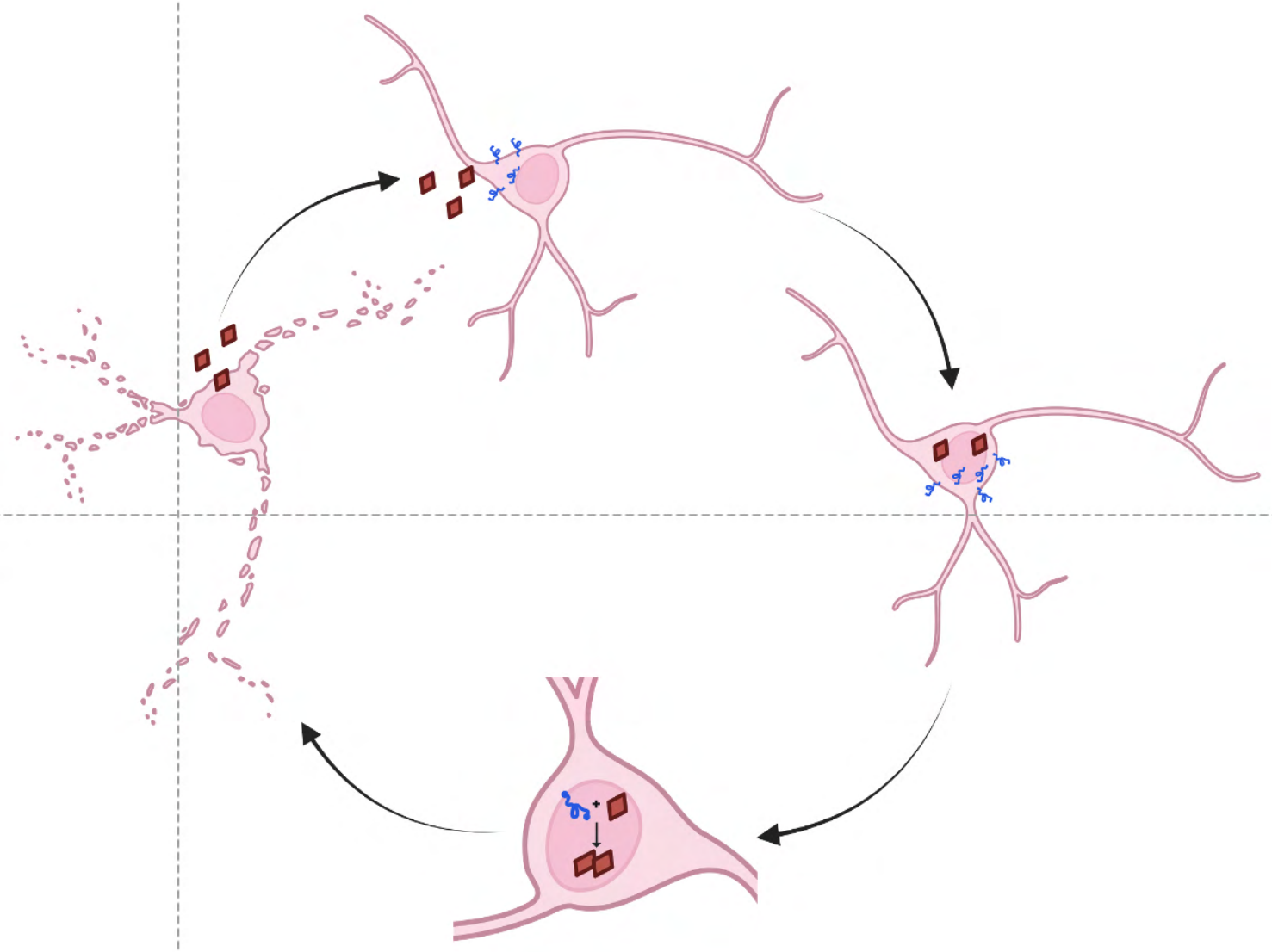


Banks, Susan ML, et al. (2020).

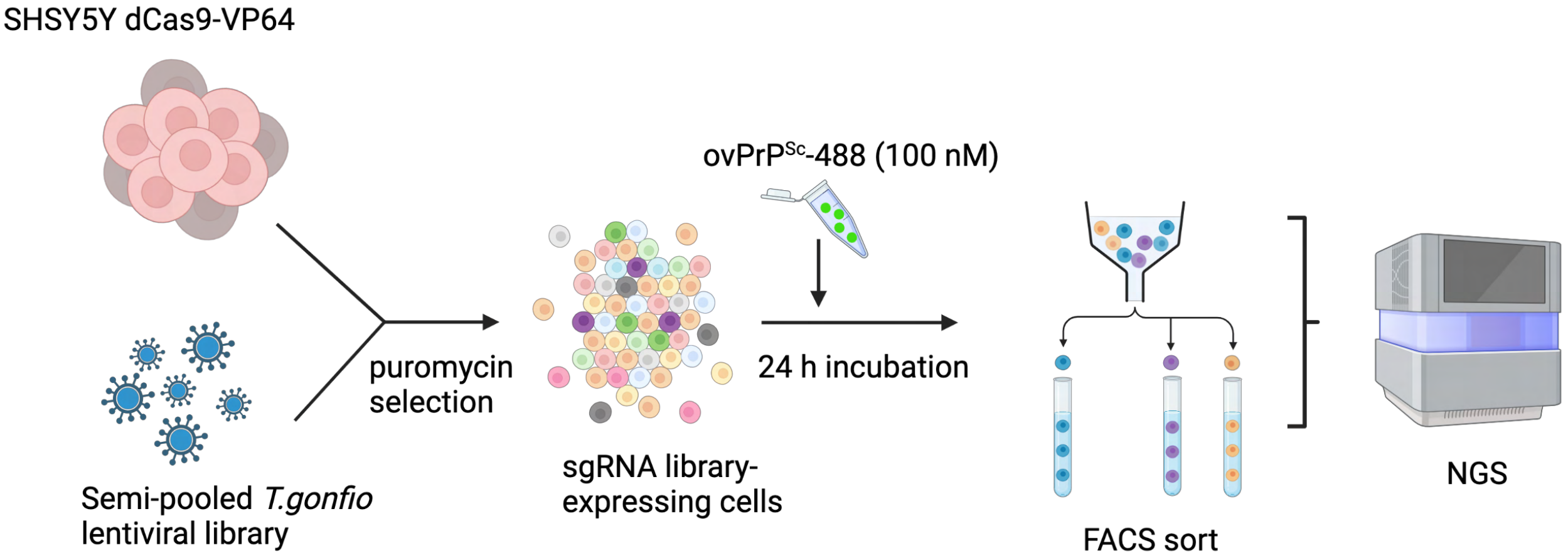
Identification of genetic modulators of prion uptake via CRISPR screens

Elena De Cecco, PhD

University of Zurich

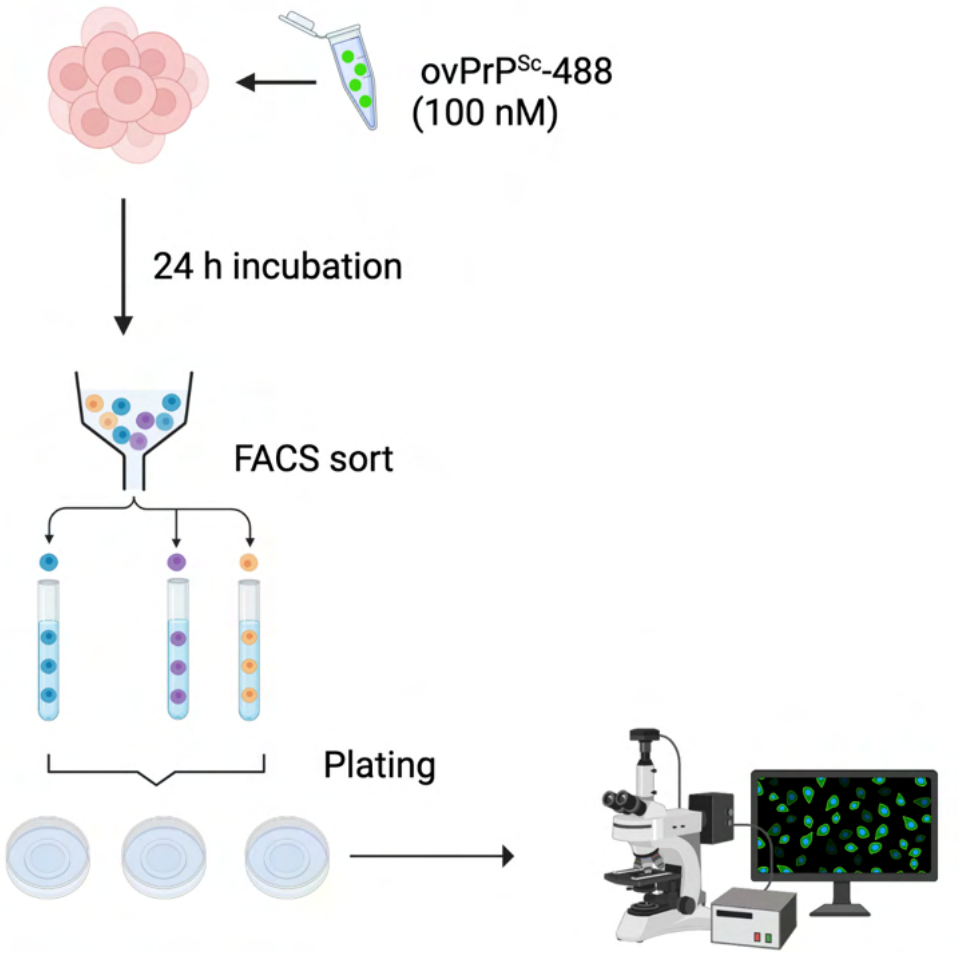


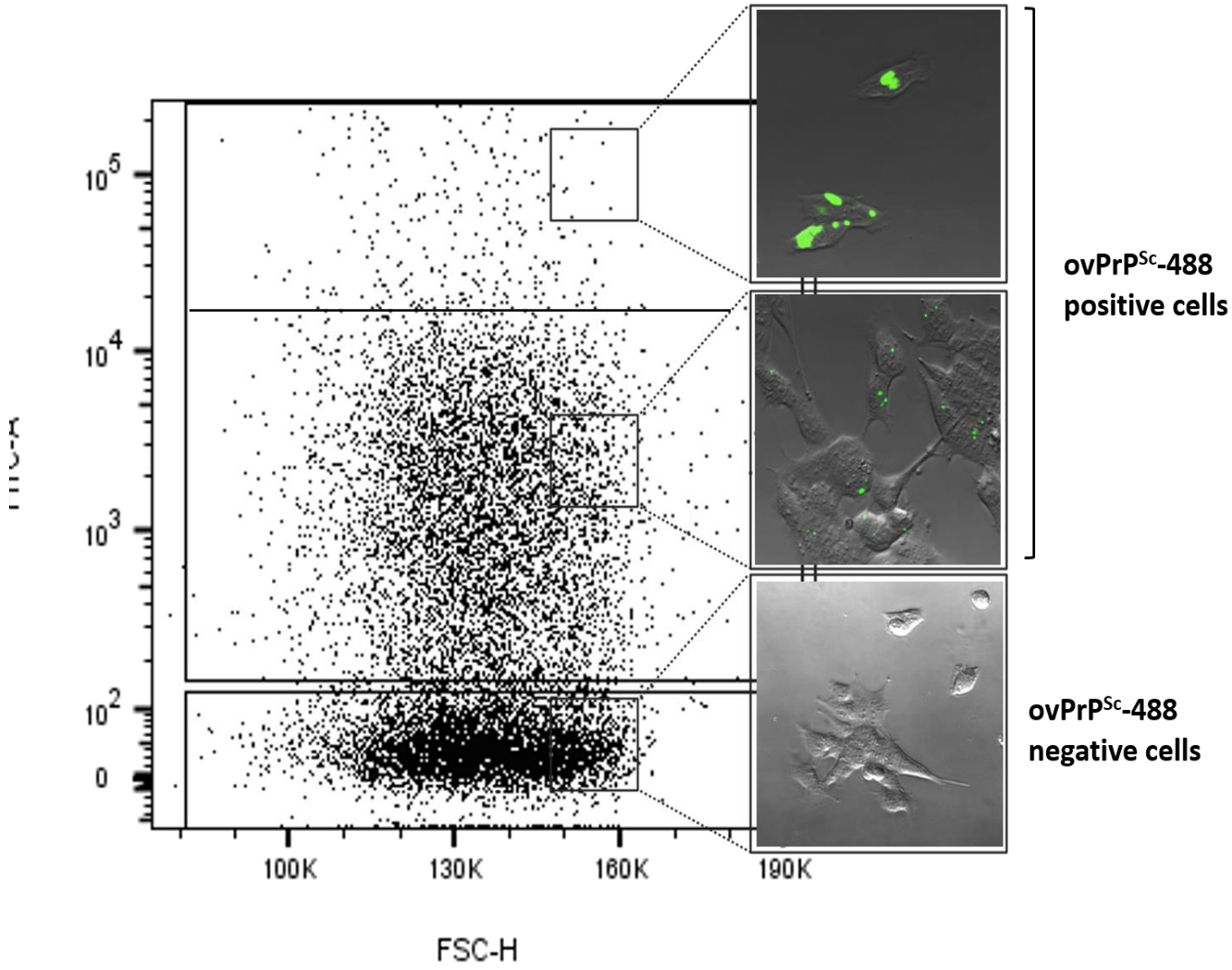
Can we identify genes that modulate prion uptake?



INTERNALIZATION CHECK

Sorting and visual inspection of sorted cells



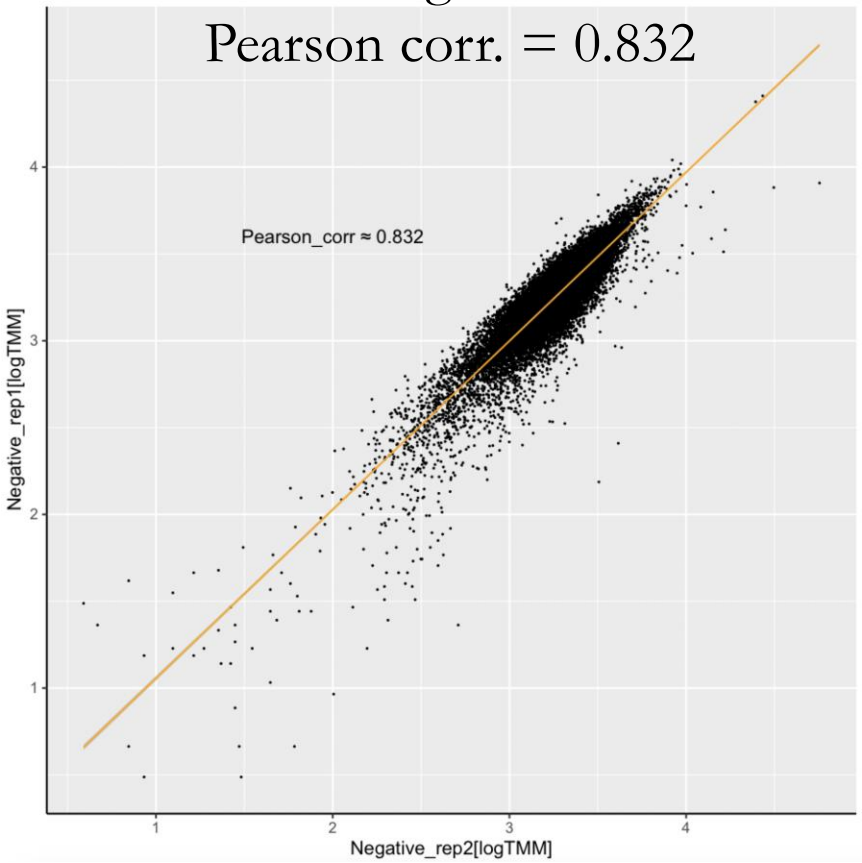


Genome–wide screen - QC

Davide Caredio

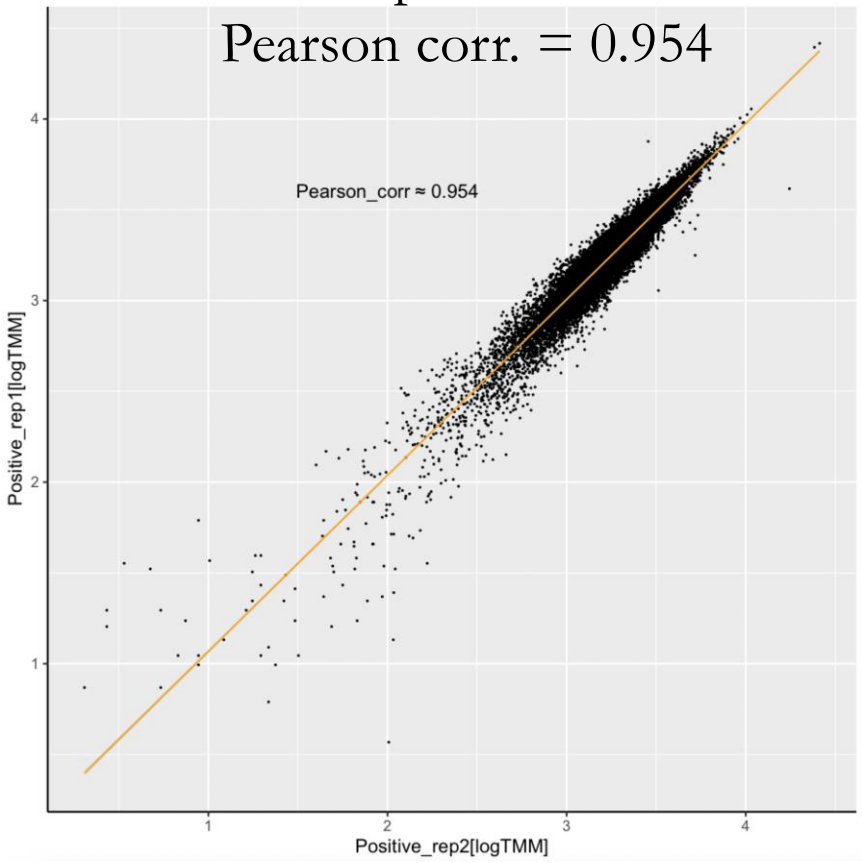
Prion negative cells

Pearson corr. = 0.832



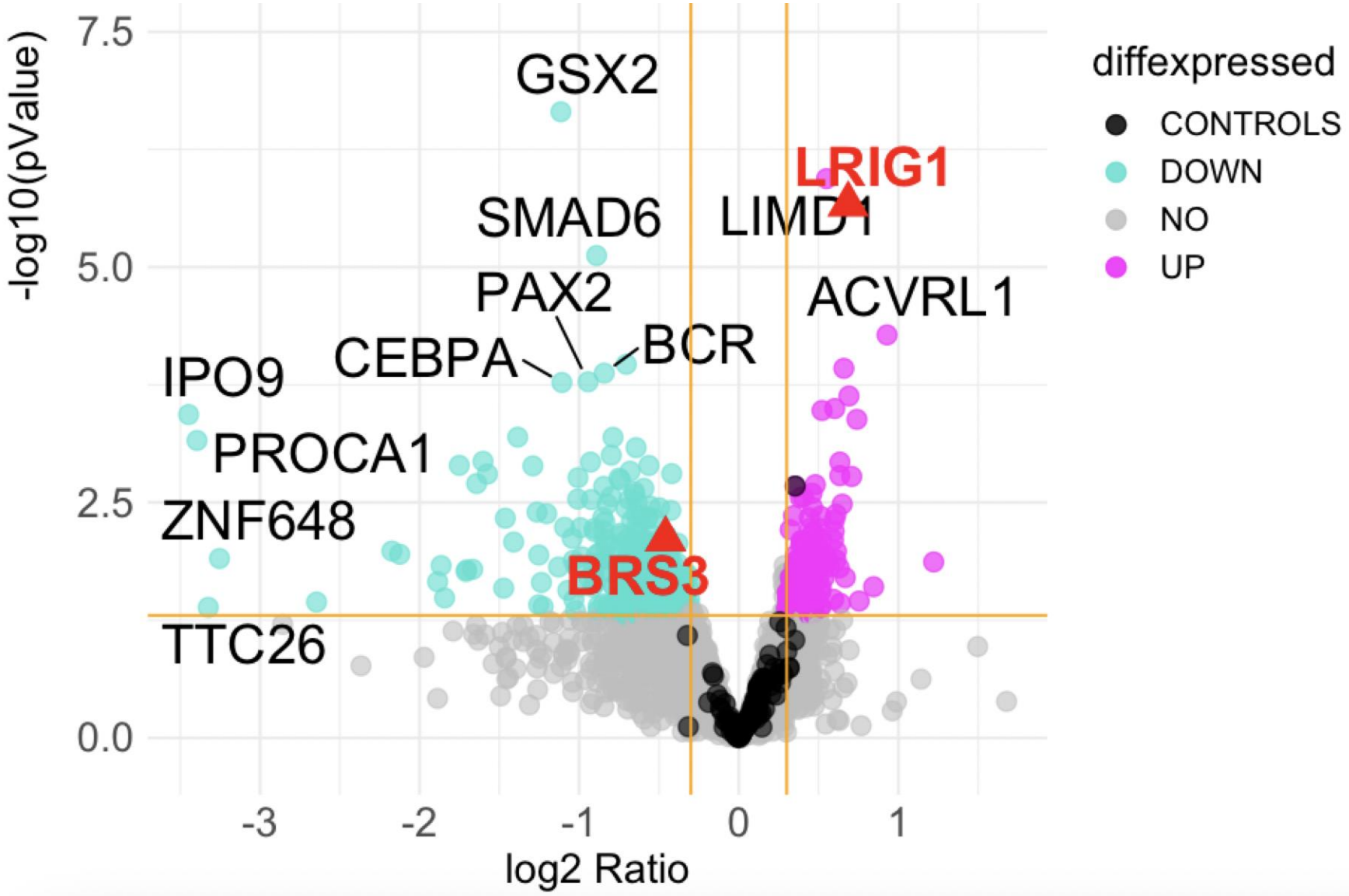
Prion positive cells

Pearson corr. = 0.954

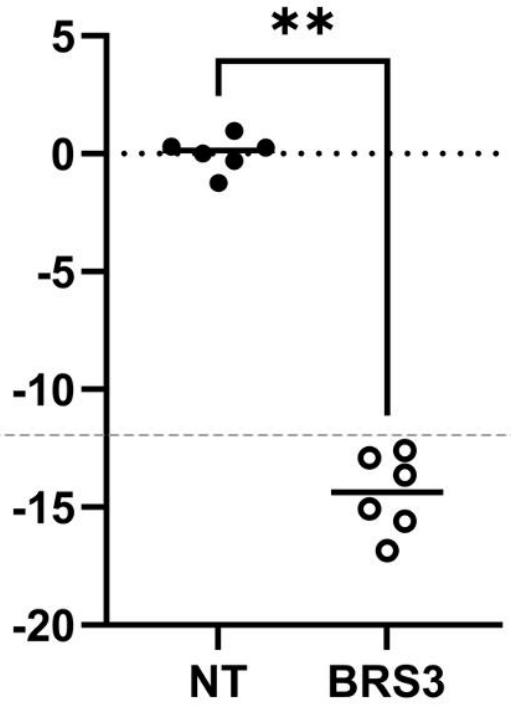


Very good replicate correlation of both prion negative and prion positive cells

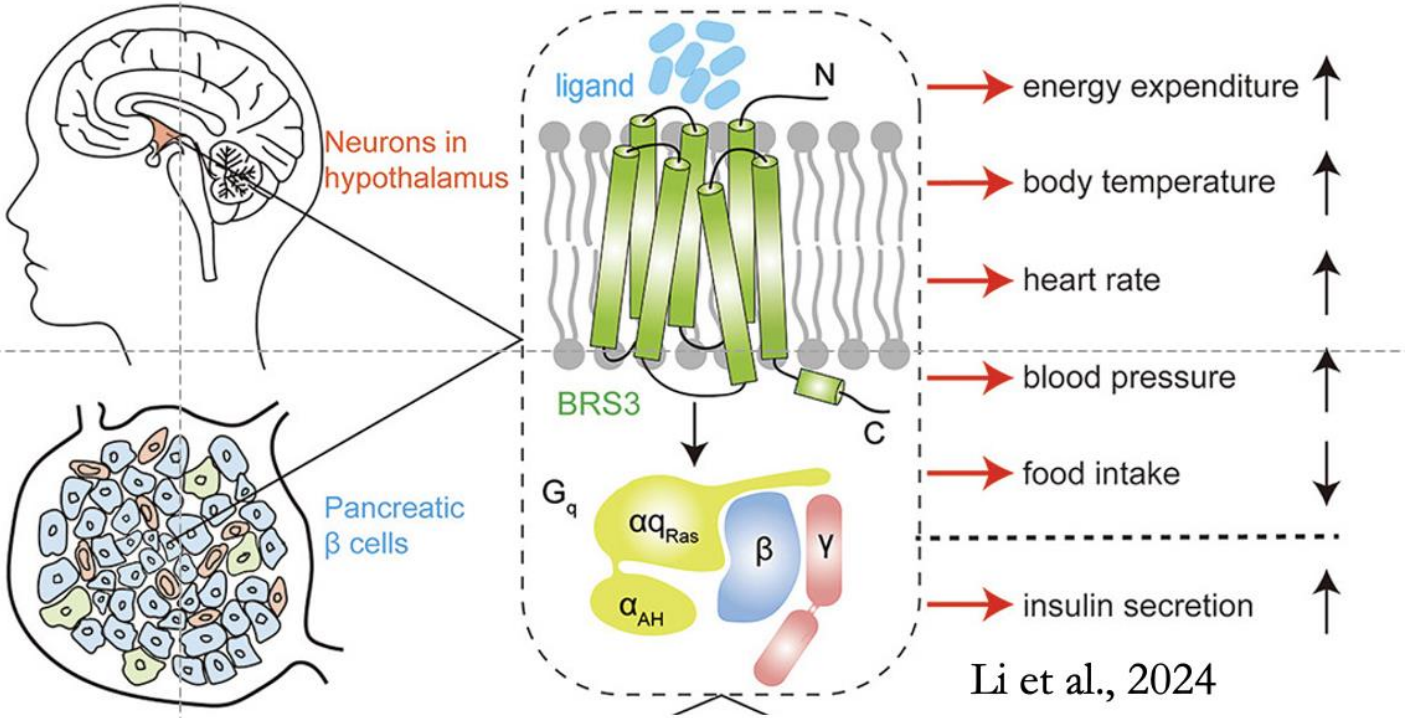
Genome-wide CRISPRa screen



AN UNEXPECTED CANDIDATE: BRS-3



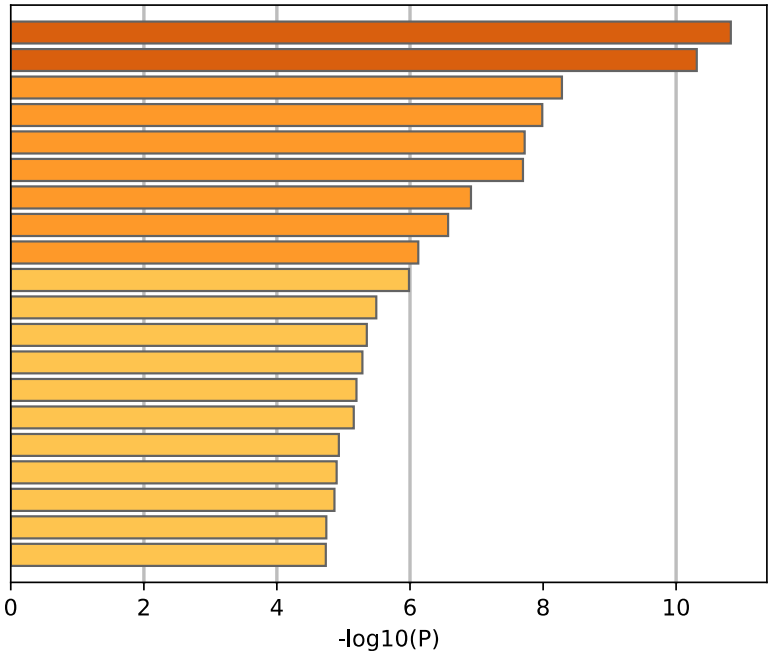
BRS3 is an orphan GPCR expressed in pancreas and in the hypothalamus



Li et al., 2024

BRS3 is poorly characterized and not associated to neurodegeneration

Pathway analysis



GO:0048598: embryonic morphogenesis
GO:0007423: sensory organ development
GO:0045165: cell fate commitment
GO:0008285: negative regulation of cell population proliferation
R-HSA-201451: Signaling by BMP
GO:0007389: pattern specification process
GO:0048705: skeletal system morphogenesis
GO:0030855: epithelial cell differentiation
GO:0060322: head development
GO:0030001: metal ion transport
GO:0001704: formation of primary germ layer
GO:0050767: regulation of neurogenesis
GO:0000165: MAPK cascade
GO:0007167: enzyme-linked receptor protein signaling pathway
GO:0031667: response to nutrient levels
hsa05202: Transcriptional misregulation in cancer
GO:0040007: growth
GO:0003197: endocardial cushion development
GO:0030858: positive regulation of epithelial cell differentiation
GO:0048608: reproductive structure development

BMP pathway identified in several databases

explain columns

Reactome Pathways

pathway	*description*	*count in network*	*strength*	*false discovery rate*
HSA-201451	Signaling by BMP	7 of 27	1.13	0.0066

WikiPathways

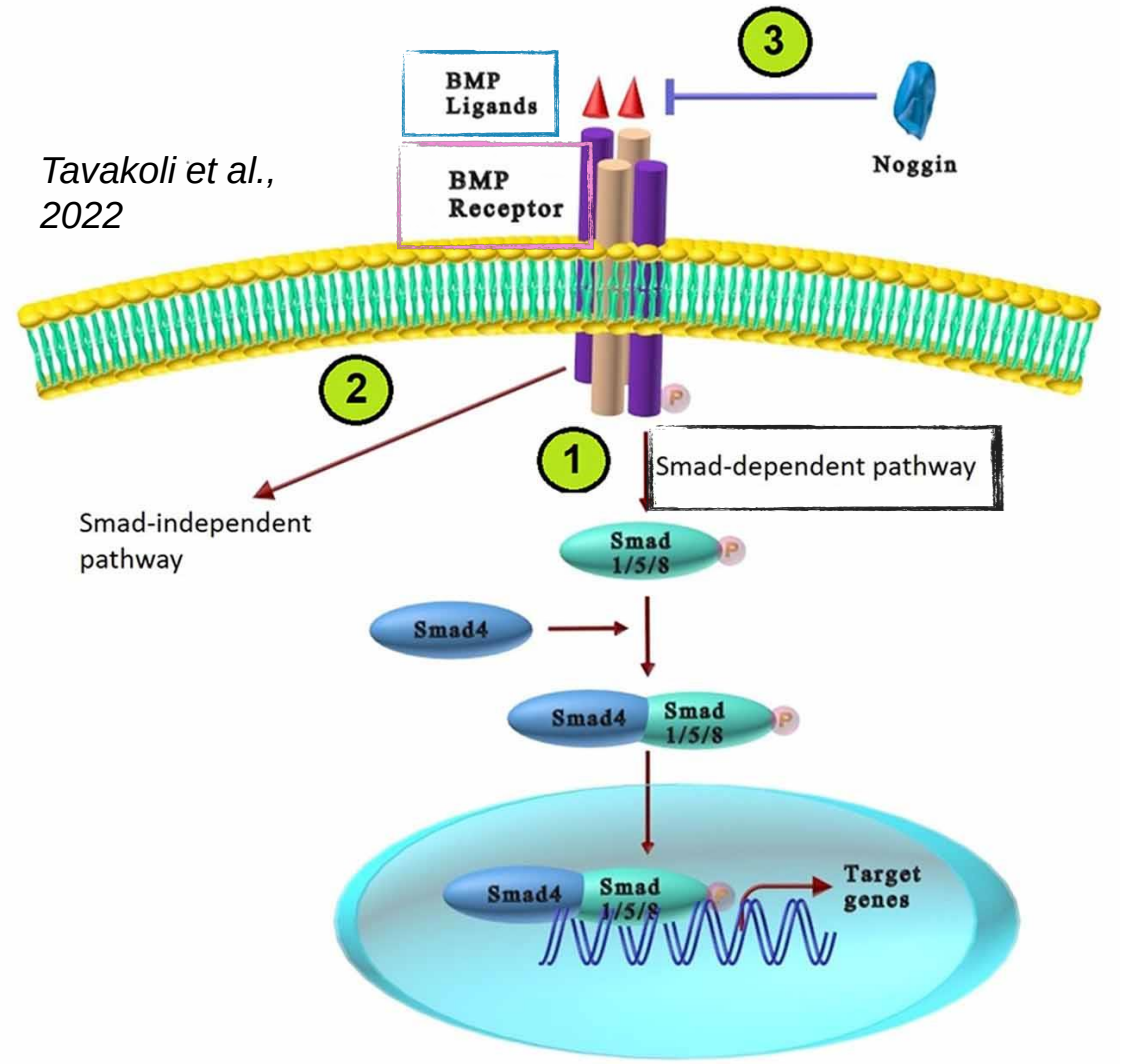
pathway	*description*	*count in network*	*strength*	*false discovery rate*
WP1425	Bone morphogenic protein signaling and regulation	5 of 12	1.33	0.0096

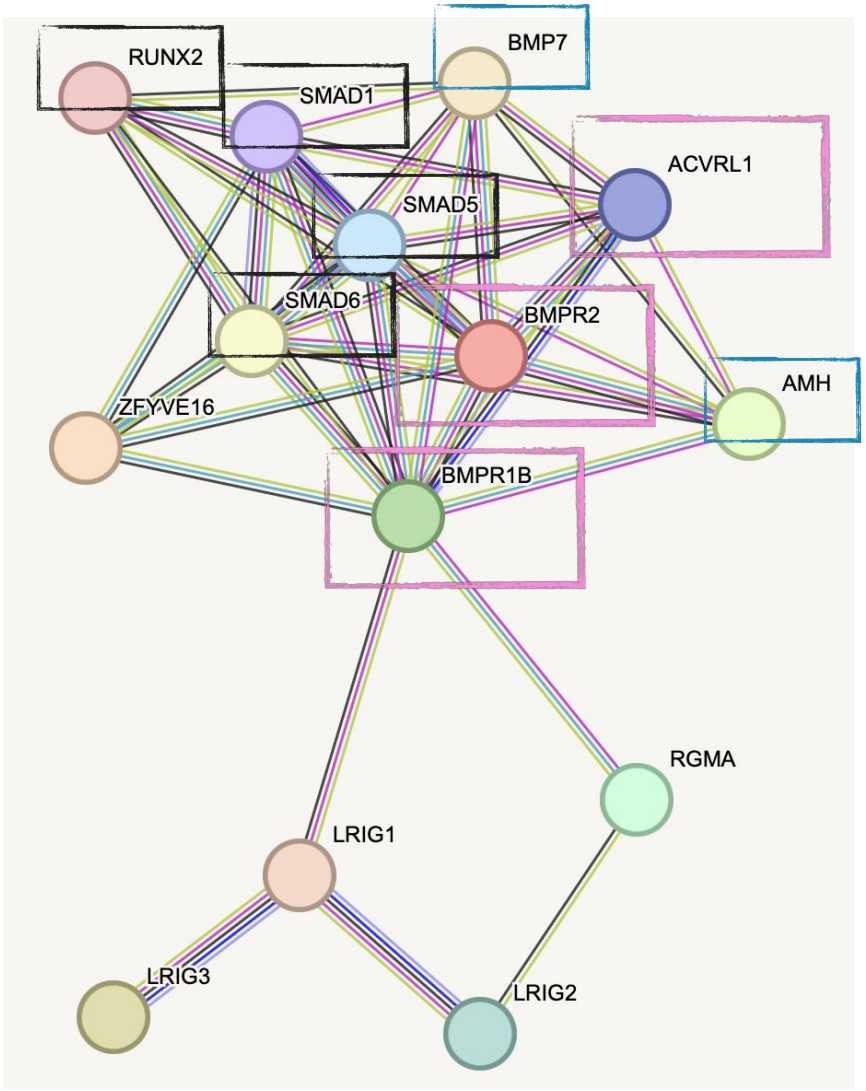
Tissue expression (TISSUES)

tissue	*description*	*count in network*	*strength*	*false discovery rate*
BTO:0000202	Sense organ	45 of 1124	0.32	0.0125

Bone Morphogenetic Protein Signalling Pathway

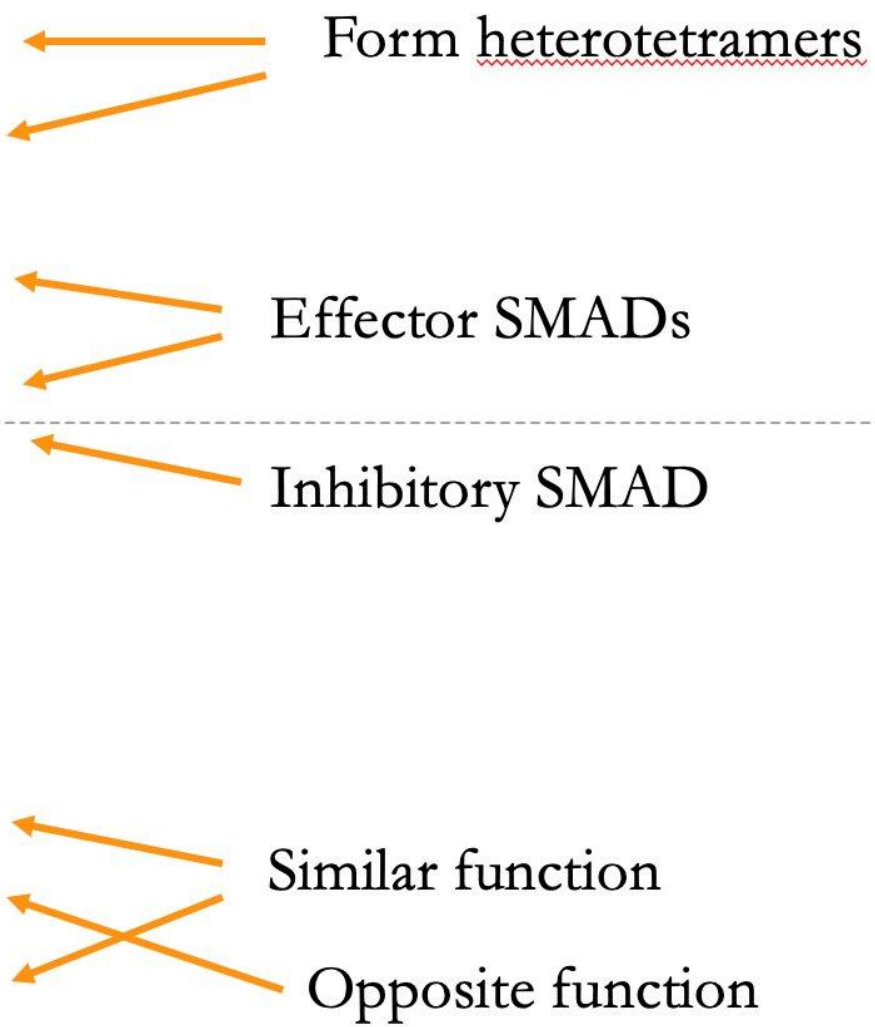
Tavakoli et al., 2022





BMP pathway – identified hits

GENE	SCORE	FUNCTION
BMPR1B	UP (21.5)	Receptor (S/T kinase)
BMPR2	UP (36)	Receptor (S/T kinase)
BMP7	DOWN (47)	Secreted ligand
SMAD1	UP (25)	Transcription factor
SMAD5	UP (20.6)	Transcription factor
SMAD6	DOWN (46)	Transcription factor
RUNX2	DOWN (37)	Transcription factor
RGMA	UP (31)	GPI protein
AMH	DOWN (31.3)	Secreted ligand
ACVRL1	UP (45.6)	Receptor (S/T kinase)
LRIG1	UP (38.7)	EGFR regulator (inhibitor)
LRIG2	DOWN (38.7)	EGFR regulator (activator)
LRIG3	UP (28)	EGFR regulator (inhibitor)



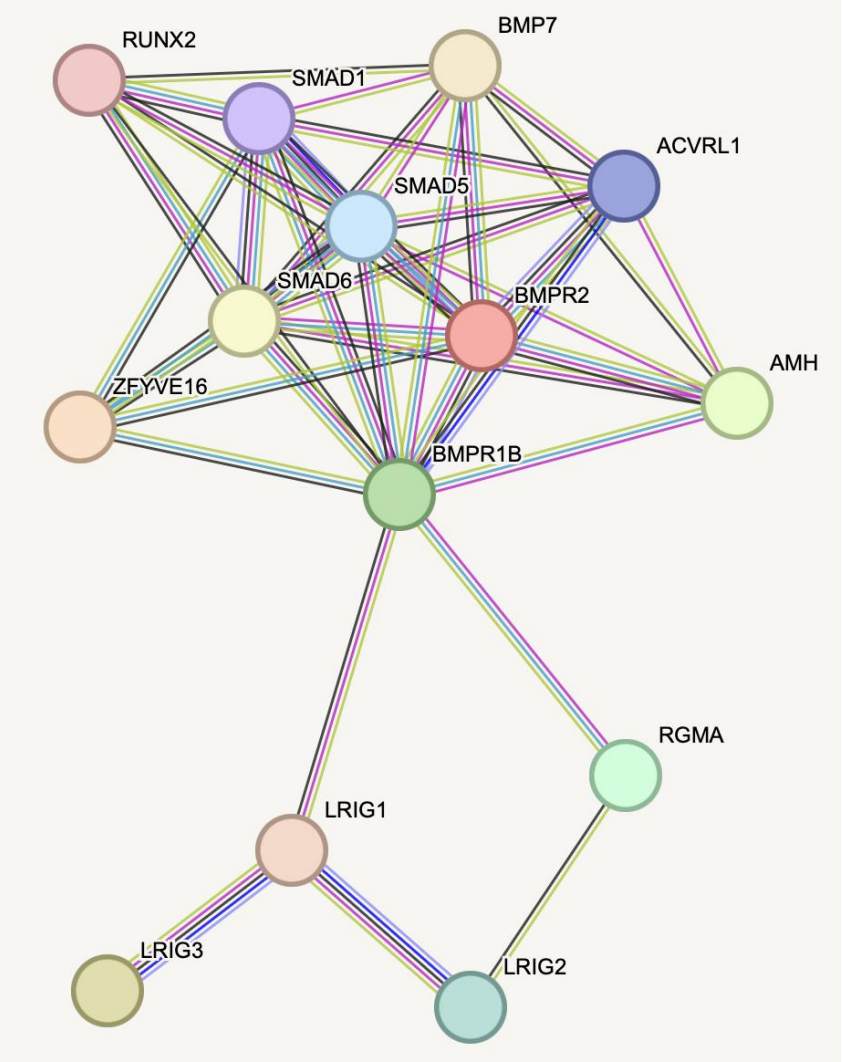
Their partners: the LRIG brothers

GENE	SCORE	FUNCTION
LRIG1	UP (38.7)	EGFR regulator (inhibitor)
LRIG2	DOWN (38.7)	EGFR regulator (activator)
LRIG3	UP (28)	EGFR regulator (inhibitor)

LRIG proteins regulate lipid metabolism via BMP signaling and affect the risk of type 2 diabetes

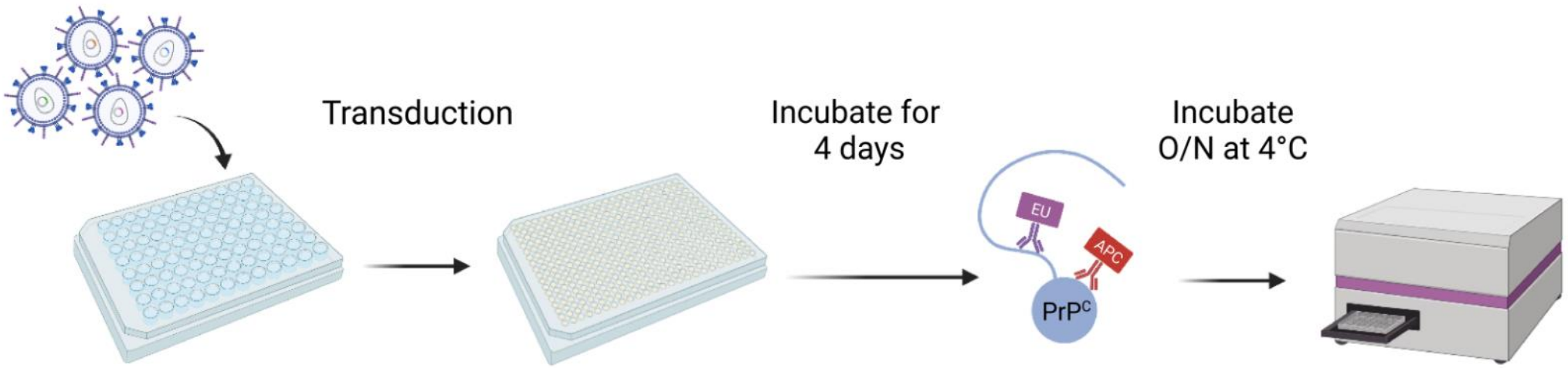
Carl Herdenberg, Pascal M. Mutie, Ola Billing, Ahmad Abdullah, Rona J. Strawbridge, Ingrid Dahlman, Simon Tuck, Camilla Holmlund, Peter Arner, Roger Henriksson, Paul W. Franks & Håkan Hedman

LRIG-KO mice are deficient in BMP signalling
Phenotype is rescued by overexpression of LRIG1 and LRIG3, but not LRIG2



Whole Genome-Wide Arrayed CRISPRa Screen to identify genetic modulators of PrP^C expression

Chiara Trevisan, Hao Wang



T.Gonfio arrayed library packaged in lentiviruses

U251-MG expressing dCas9-VPR grow in individual wells

Discard medium
Add lysis buffer
Add TR-FRET antibodies

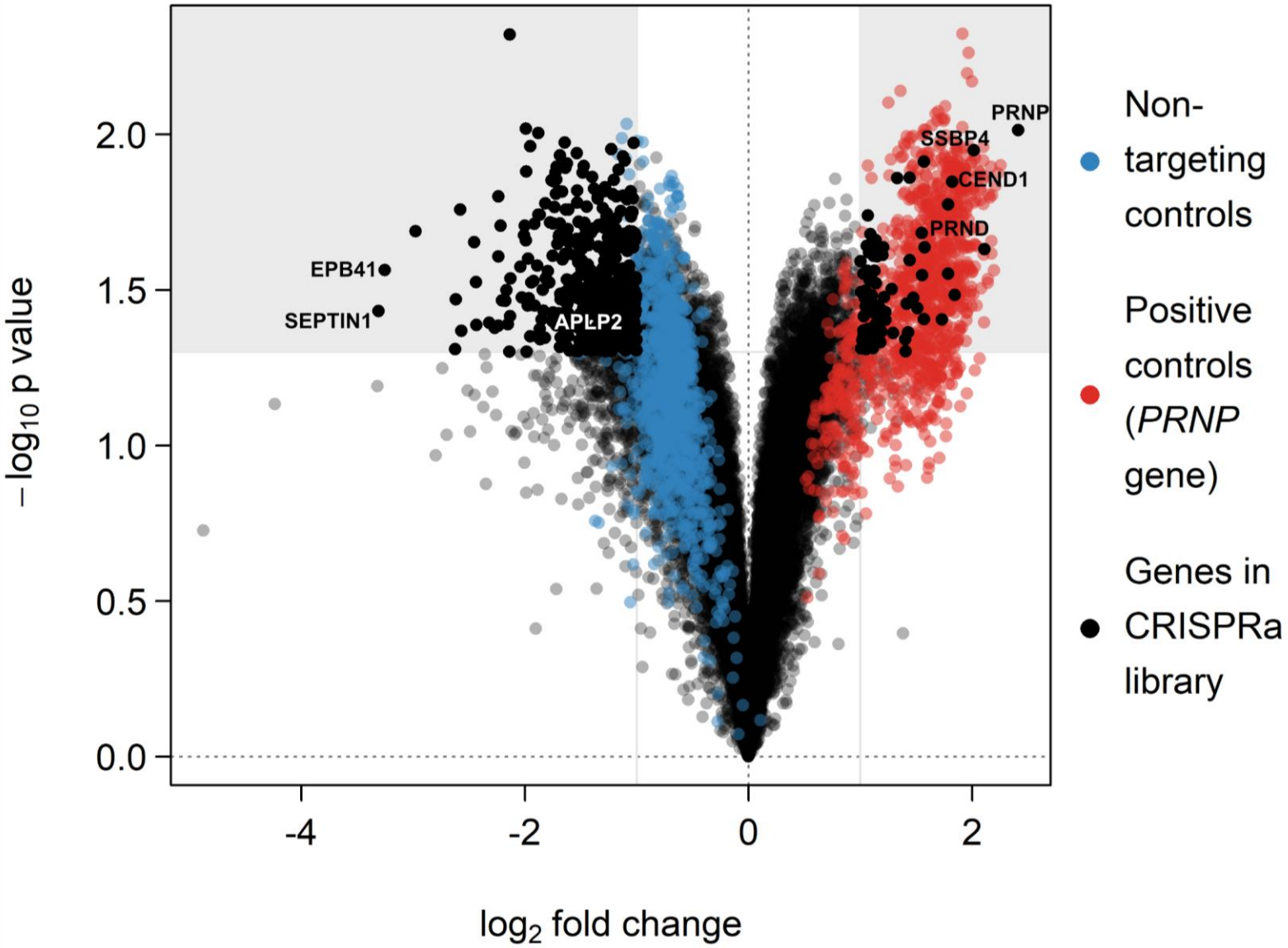
Read TR-FRET

Selection of candidate genes: normalization by the median of the genes

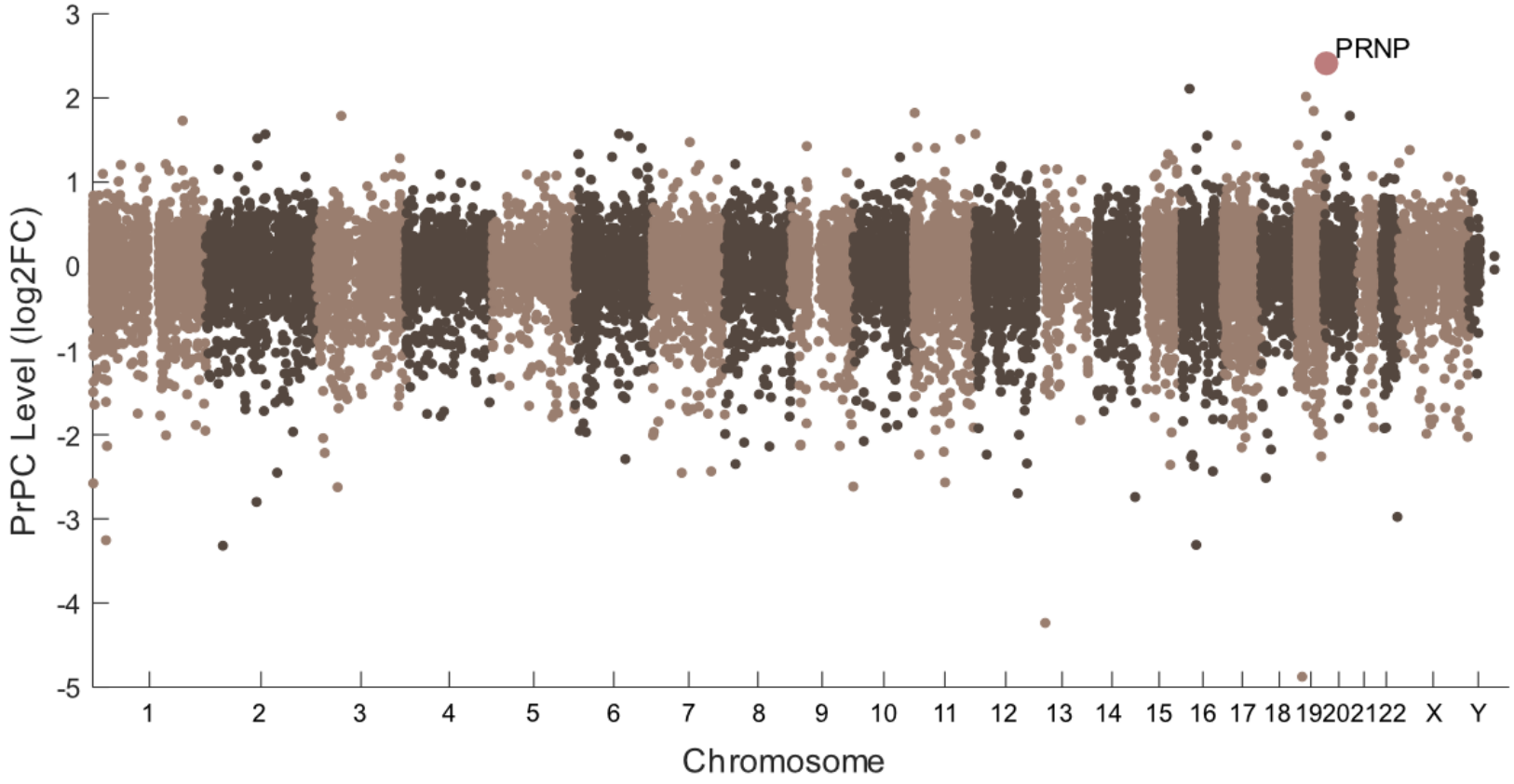
Cut-off criteria: p-value <0.05 Fold change: <0.5 or >2 (= $\log_2$FC < -1 or >1)

80 Upregulators

451 Downregulators



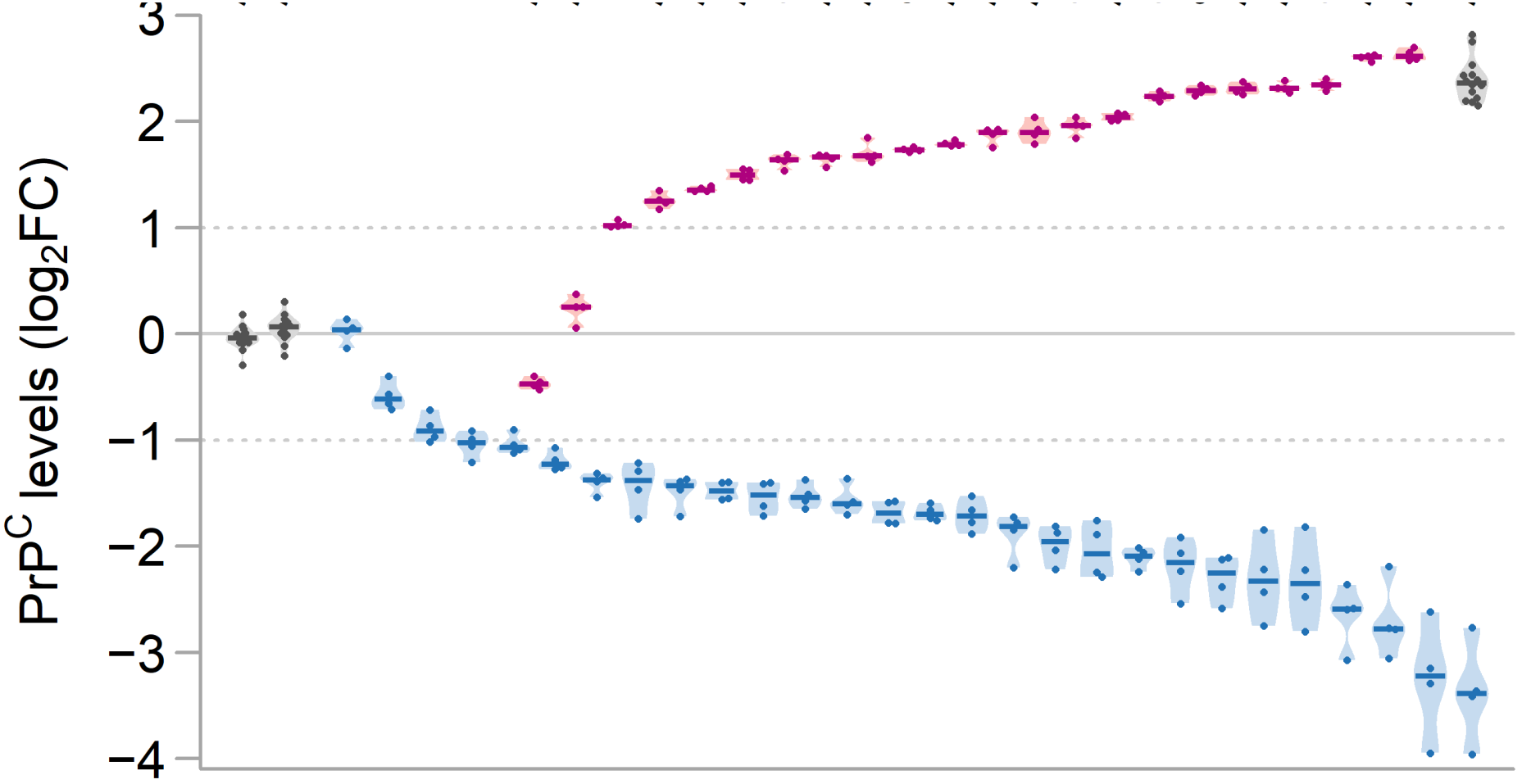
Physical location of PrP^C upregulators



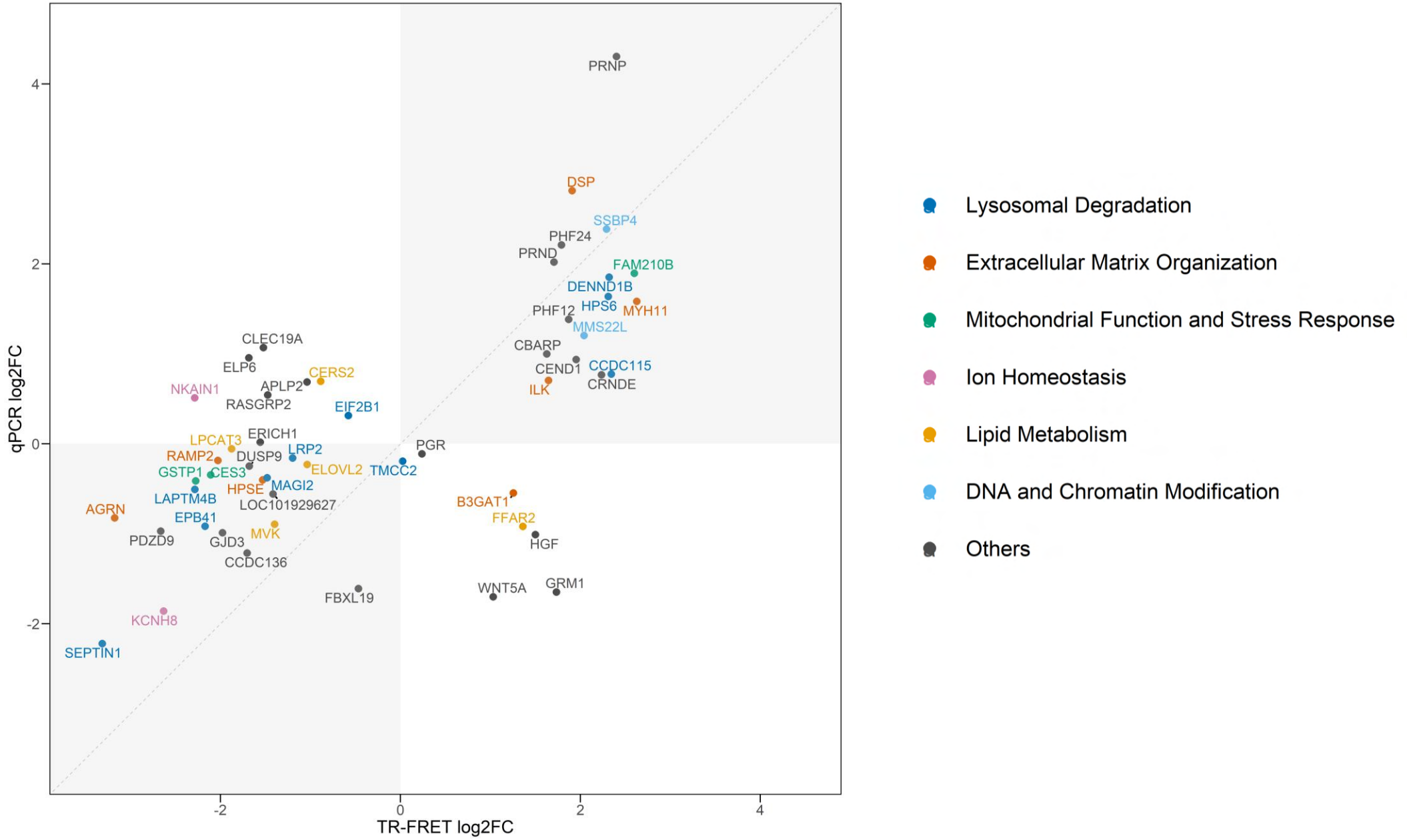
TR-FRET to reproduce the results and exclude false positive

4 replicates per gene

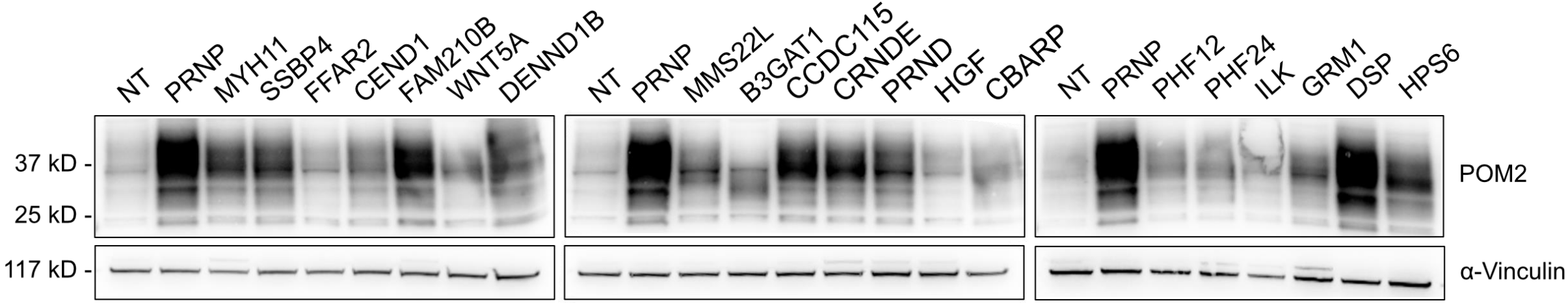
Cut-off criteria: p-value <0.05 Fold change: <0.5 or >2 (= $\log_2$FC < -1 or >1)



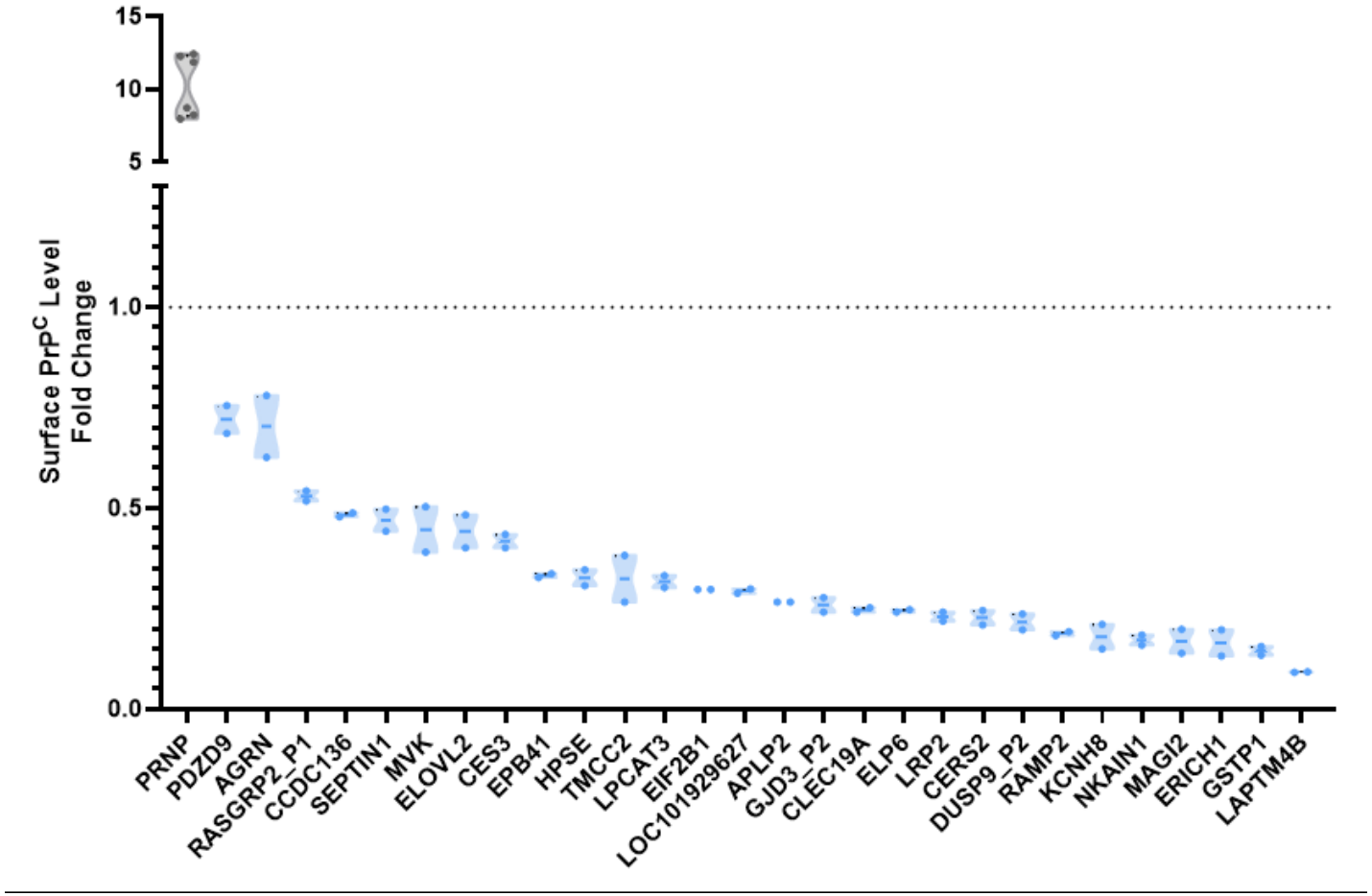
Transcriptional vs. post-transcriptional modifiers

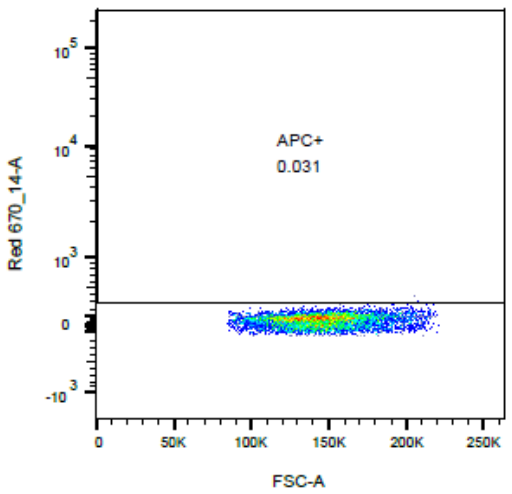


Hit validation of PrP^C upregulators by Western blotting

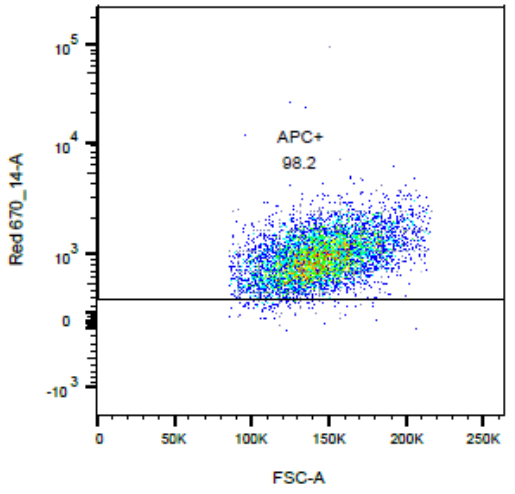


Hit validation of PrP^C downregulators by flow cytometry

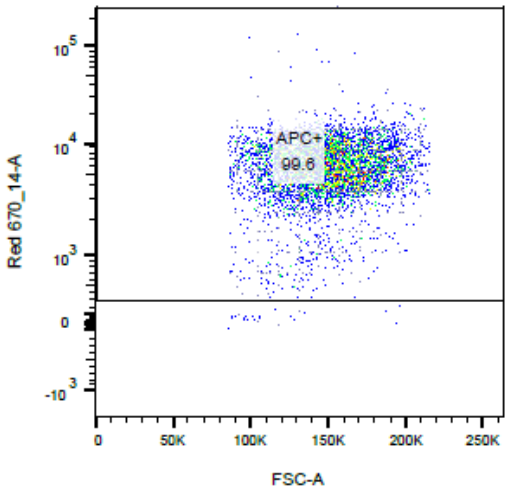




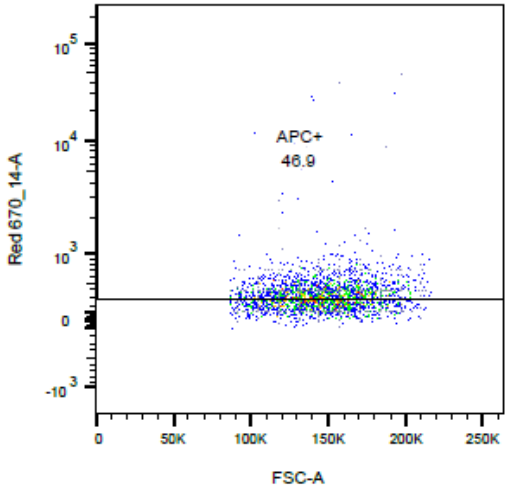
No Stain



Wild-type



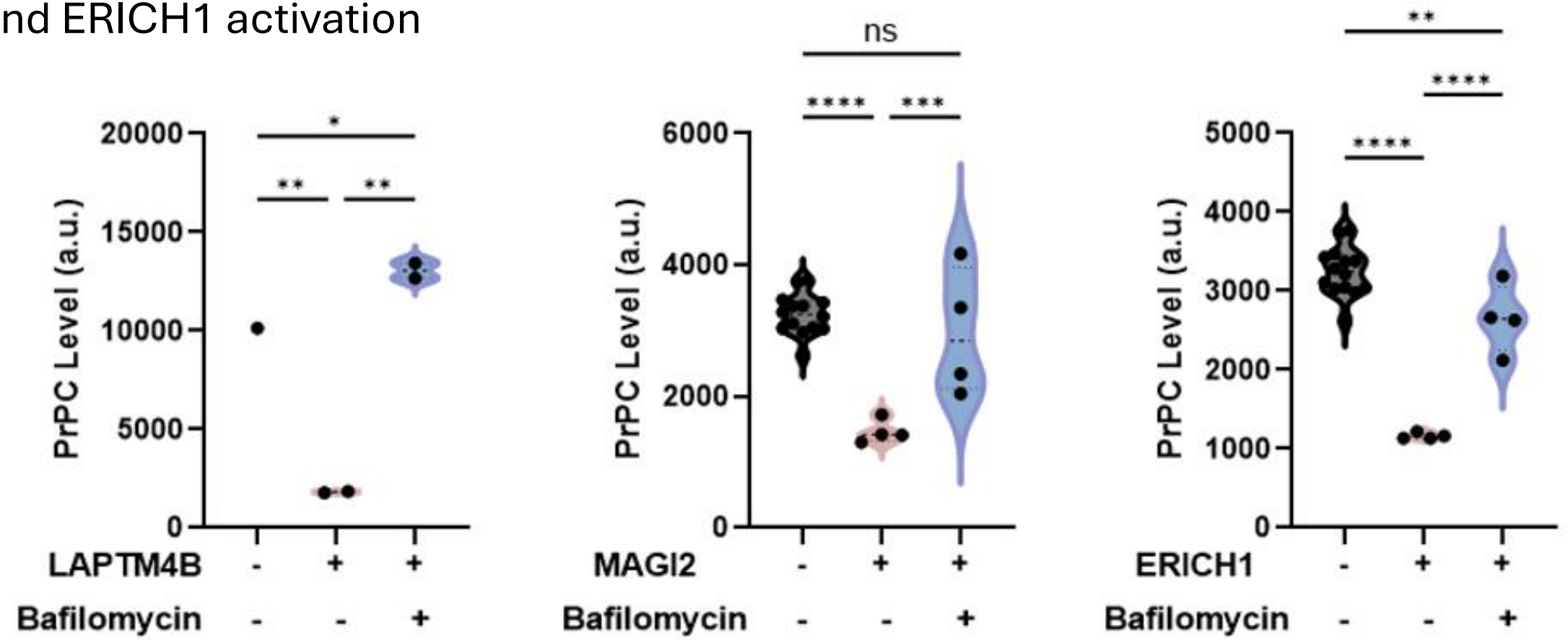
PRNP



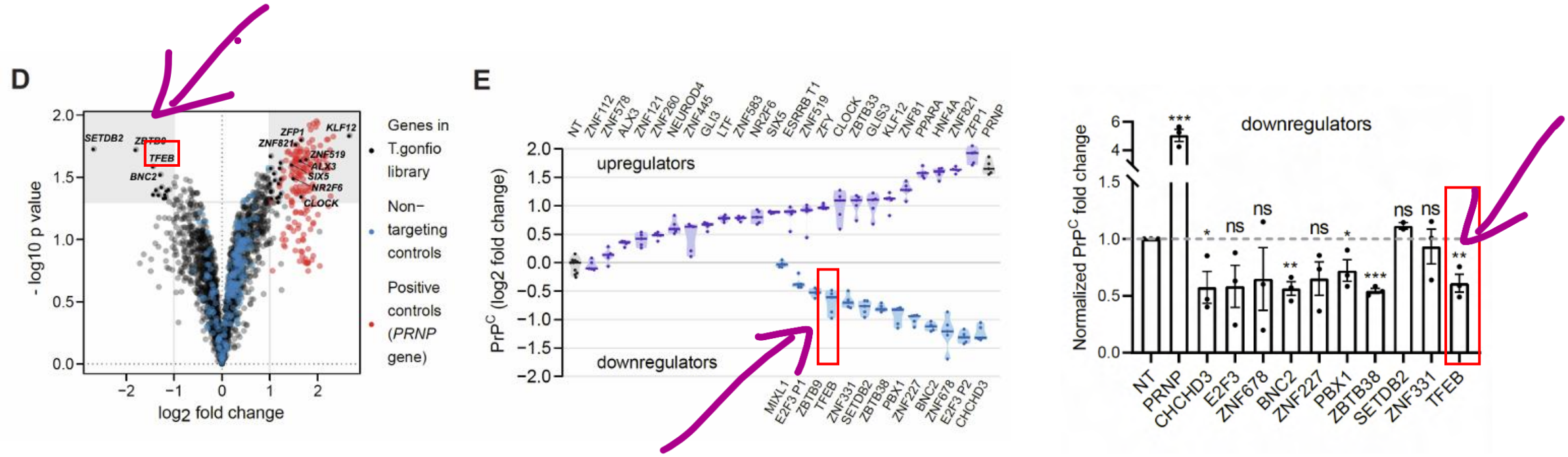
LAPTM4B

Some of the strongest downregulators of surface PrP^C are involved in lysosomal degradation

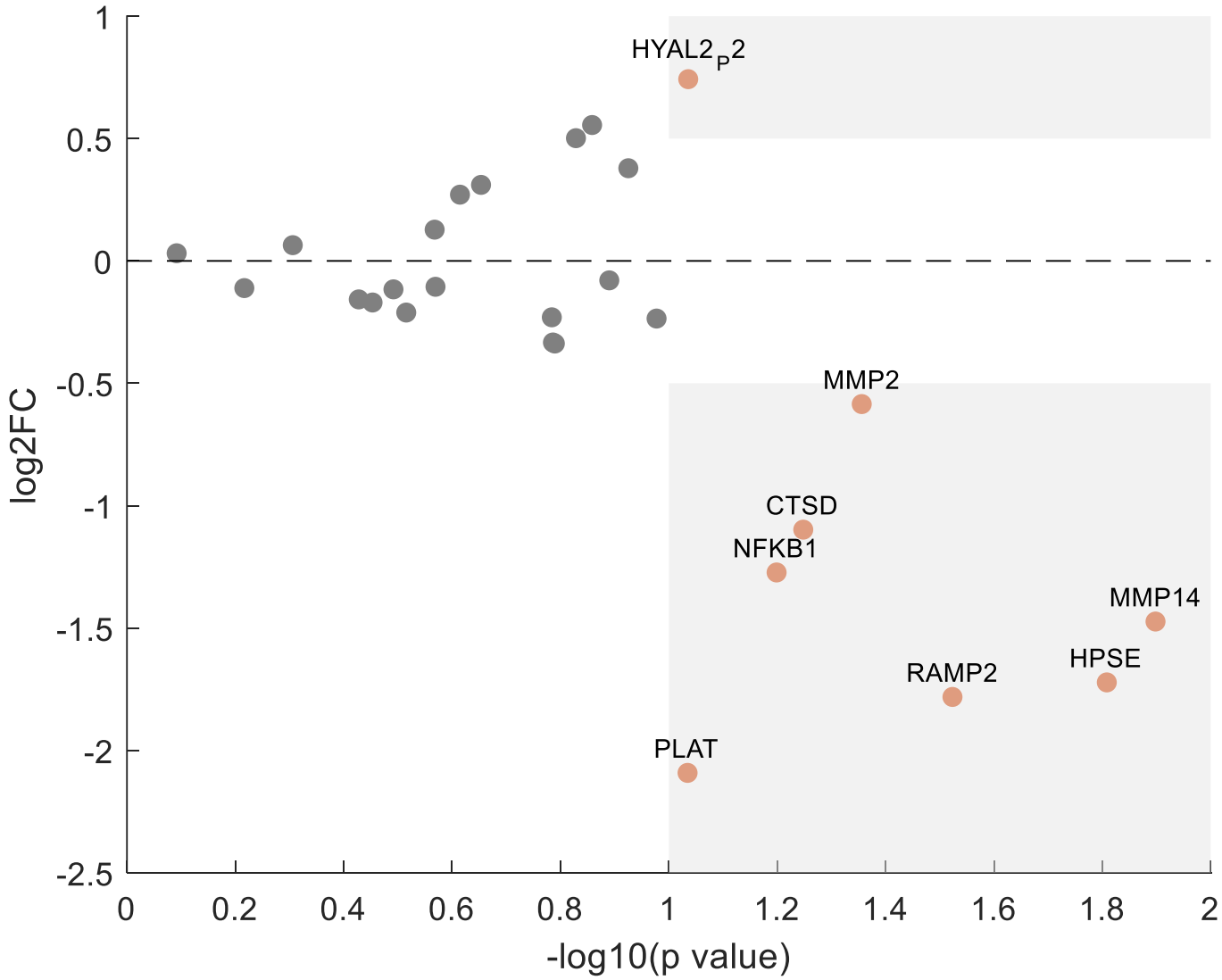
- LAPTM4B is a lysosomal protein that may mediate the transport of PrPC into lysosomes
- MAGI2 is involved in endocytosis and may perhaps mediate PrP^C endocytosis
- Bafilomycin inhibits lysosomal function and suppresses the effect of LAPTM4B, MAGI2 and ERICH1 activation



TFEB, a master regulator of lysosomal biogenesis and autophagy, showed up as a PrP^C downregulator.



Several genes involved in ECM breakdown downregulate PrP^C




CRISPR4ALL
Research with advanced CRISPR libraries

When all you have is a CRISPR library…

….everything looks like a screenable phenotype

We are looking for partners! If you have an informative, screenable phenotype, and are willing to apply jointly for competitive funding, you know where I live.