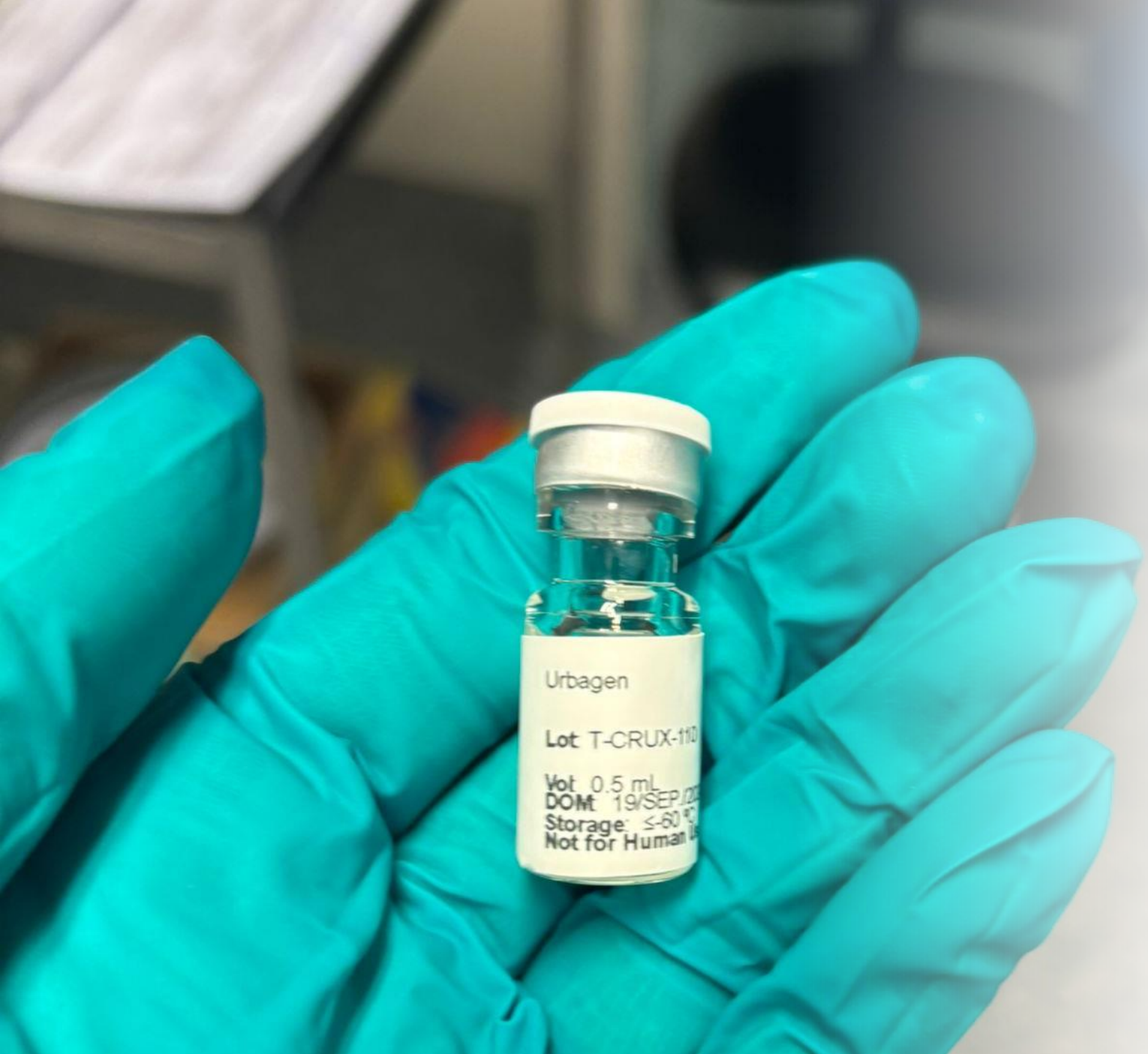


CTNNB1 FOUNDATION
Bringing hope to children

CTNNB1 GENE THERAPY: UPDATE ON DEVELOPMENT AND CLINICAL TRANSLATION

Dr. Špela Miroševič

CTNNB1 Foundation, The Gene Therapy Research Institute

About

CTNNB1 FOUNDATION
Bringing hope to children

Establishment

•Established in February 2021 and obtained consent of the Ministry of Health of Slovenia

•Officially registered as non-profit institution on the 7th of March 2021.

Mission

• Develop and support treatment solutions that have a good chance to led to the clinics

• Built knowledge about the disease and improve standards of care

• Connect researchers and families around the world

Main accomplishments:

- Raised more than 4 million euros via various fundraising events
- Obtained exclusive license from CMRI and initiated manufacturing process
- Amended Slovenian law for Slovenian government to support early gene therapy programs
- Organized three international conferences, and two NHS (Slovenia, Spain, Australia, USA, Canada)
- Granted Orphan Designation for the product URBAGEN (EMA/PM/0000262670)

CTNNB1 team

CTNNB1 FOUNDATION
Bringing hope to children

LEADING TEAM

- Špela Miroševič, PhD, Founding President
- Samo Miroševič, Co-founder
- Lavra Debeljak, Research and Patient Relations Associate
- Bruno Ramalho, Technical Director
- Ana Gonzalez Hernandez, PhD, Estibaliz Martin Medina, Association CTNNB1 Spain

fundación columbus

PATIENT-DRIVEN ORGANISATION SUPPORT

- Terry Pirovalski, Elpida & SPG50
- Julia Taravella, Rare Trait Hope Fund
- Pat Furlong, Parent Project Muscular Dystrophy (PPMD)
- Amber Freed, SLC6A1 Connect

FAMILY NETWORK

- Effie Parks, Family Network Liason
- Sneha Kranthi, Regulatory Expert
- Mirela Ferraro, Italian Network
- Katharin Wisniewski, German Network
- Lucy Mort, Australian Network
- Emilie Francisci, French Network

FUNDRAISING TEAM

- Larisa Štoka, Palčica Pomagalčica
- Katarina Podgajski, Palčica Pomagalčica
- Ratko Stojiković, journalist and cameraman

INDEPENDENT CONSULTANTS

- Shivang Khandelwal, IOCB Prague
- Rodney Samaco, AUCDS
- Ruud Bueters, 3D-PharmXchange
- Basel Assaf, Tassaro LLC
- Elise Destree, 3D-PharmXchange
- Joy Cavagnaro, Access BIO

MEDICAL & SCIENTIFIC ADVISORY TEAM

CLINICAL TEAM

- Damjan Osredkar, MD, PhD, Nina Žakelj, MD, Peter Spazzapan, MD, University Medical Centre Ljubljana
- Mojca Žagar, Petra Pohleven, Alenka Piskar, University Medical Centre Ljubljana
- Laurent Servais, MD, PhD, Fiona Moultrie, MD, PhD, Charlotte Lilien, University of Oxford
- Michelle Ferrar, Sydney Children's Hospital
- Amaia Lasa Aranzasti, Hospital Universitari Vall d'Hebron, Barcelona
- Mercè Pallares Sastre, Maitane García Martín, University Deusto, Bilbao
- Sofia Montenegro, Joanna Wawrzyniak, Barbara Eva Jasinska

CLINICAL TRIAL CRO

- Mario Hercezi, Tamara Odar, Klavdija Škrlep, ADAX, International Clinical And Regulatory Organization

VECTOR DEVELOPMENT & INITIAL PRECLINICAL TESTING

- Leszek Lisowski, PhD and Andrea Perez-Iturralde, PhD , Children's Medical Research Institute

PRECLINICAL TESTING

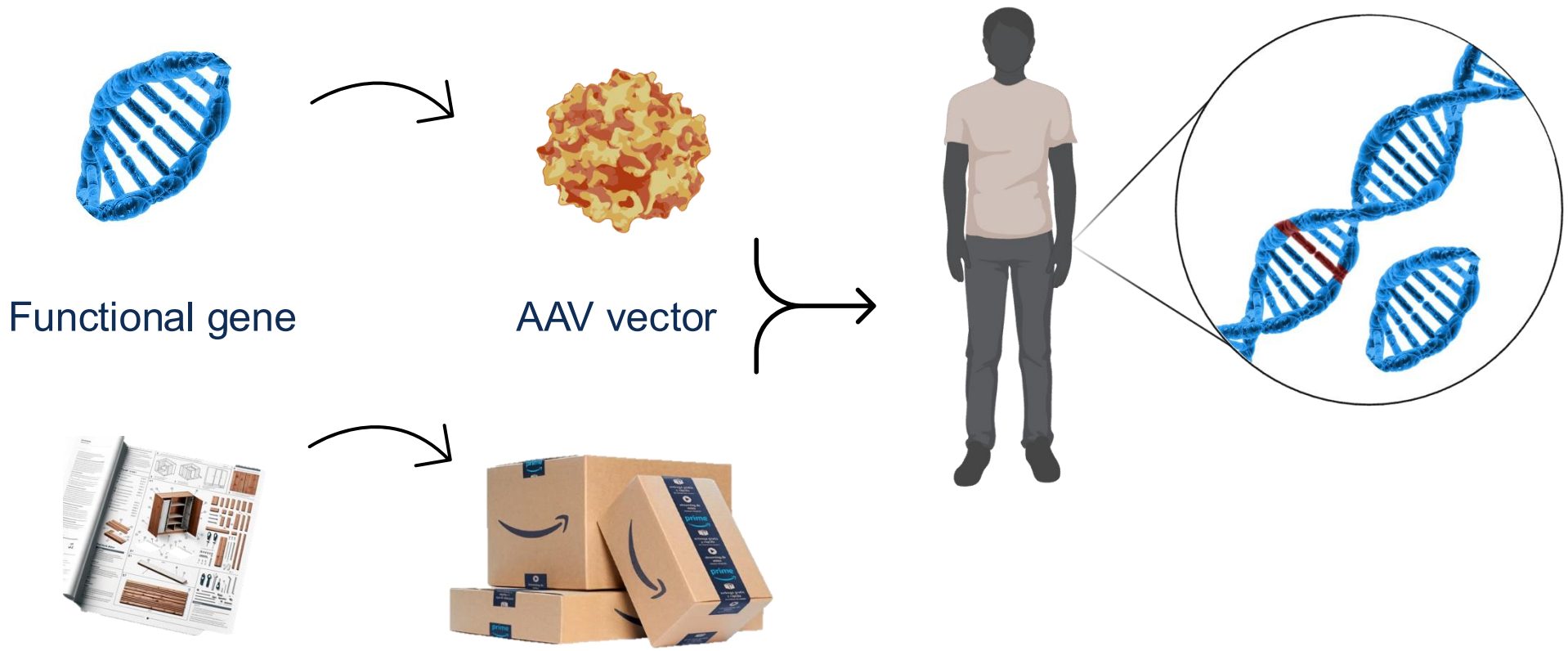
- Duško Lainšček, PhD, National Institute of Chemistry
- Vida Forstnerič, PhD, National Institute of Chemistry
- José Luis Lanciego Pérez, PhD, CIMA
- Jan Procházka, Eva Štefancova, CCP
- Uršula Prosenc Zmrzljak, PhD and Estera Merljak Zupančič, PhD, Labena

PRODUCT MANUFACTURING & LABELING

- Cristina Martin Quintin, Viralgen Vector Core
- Sonya Banks, Viralgen, Vector Core
- Almac Group Limited

What is gene replacement therapy?

Deliver functional *CTNNB1* gene to restore β-catenin expression and function



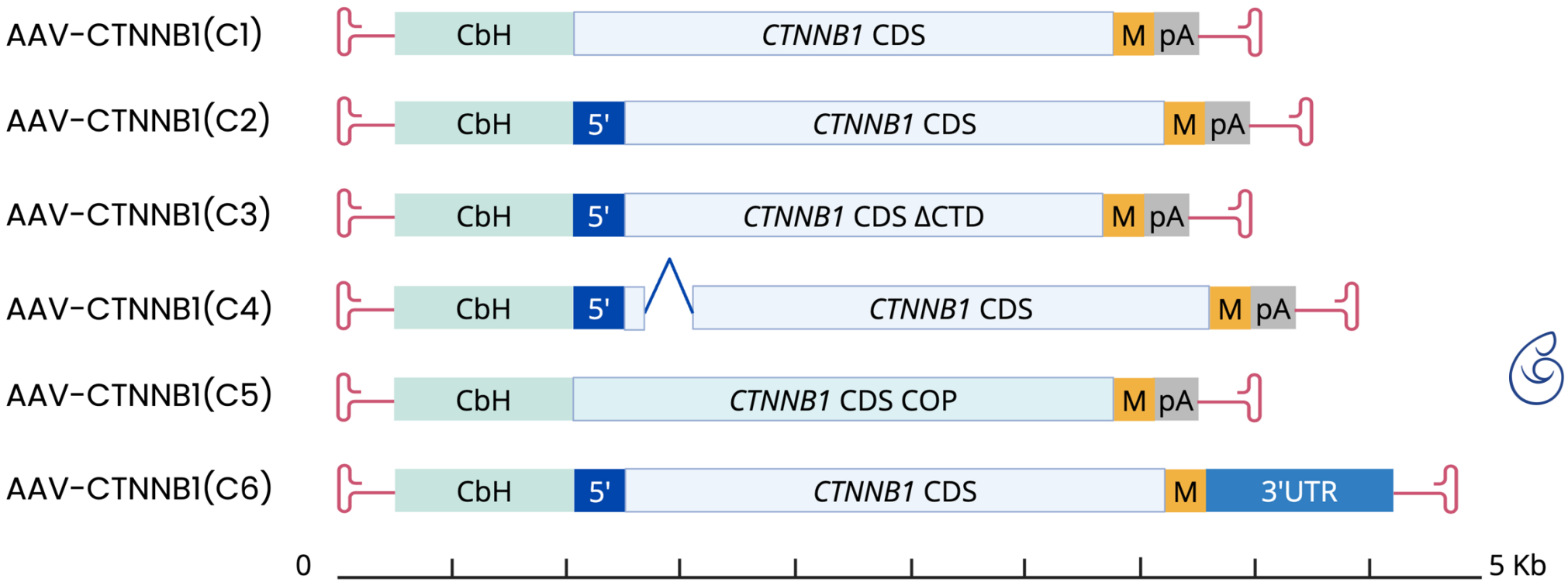
Why is gene therapy a good option for CTNNB1 syndrome?

- CTNNB1 syndrome affects the CNS
- Main cells involved are excitatory and inhibitory neurons
- CTNNB1 syndrome is caused by haploinsufficiency - meaning one working copy of the gene isn't enough. → adding a healthy copy can restore function.
- The CTNNB1 gene is small enough to fit into the AAV vector, which is essential for successful delivery.
- This approach is designed to be a "one-time" or potentially curative treatment, where the body may continue making the missing protein long-term after a single dose.

AAV-mediated gene therapy for CTNNB1 Syndrome

CTNNB1 FOUNDATION
Bringing hope to children

Functional copy of the gene accompanied by different combinations of regulatory elements:

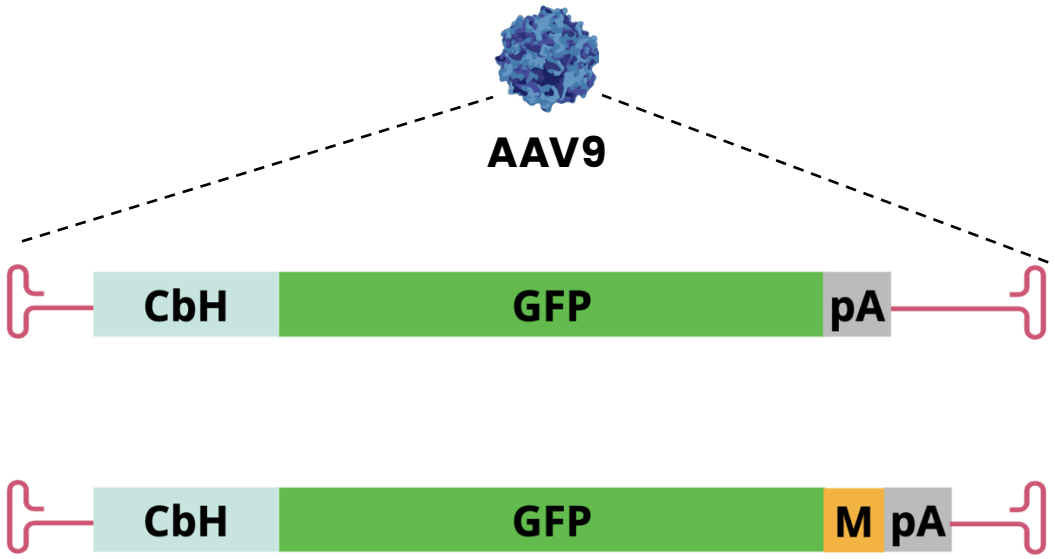


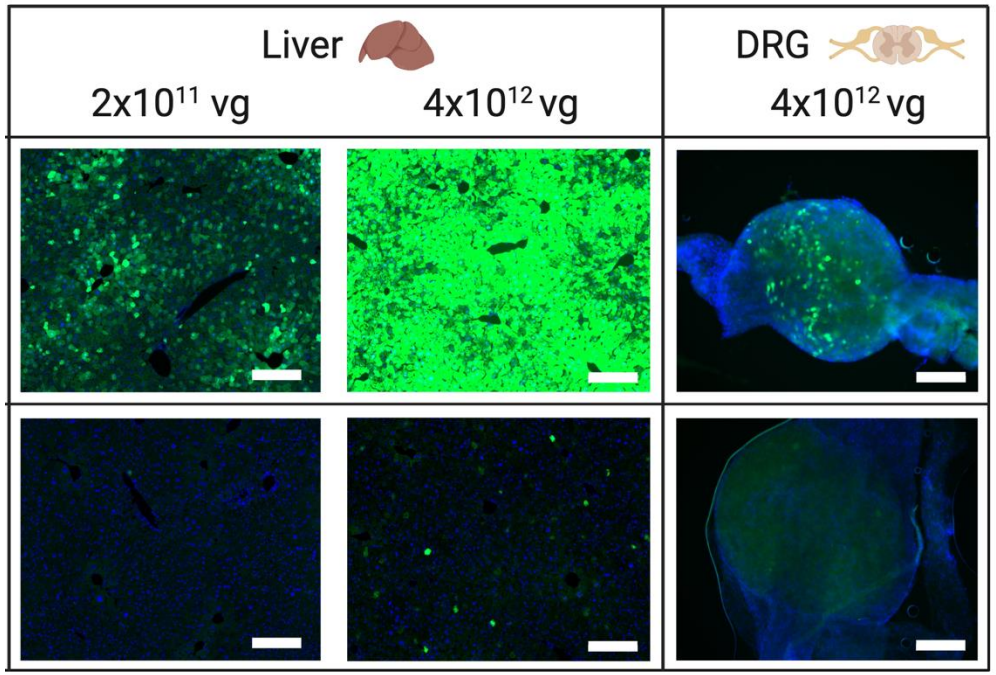
CHILDREN'S MEDICAL RESEARCH INSTITUTE

Jeans for Genes

Improving the safety profile of the therapy

MicroRNA targeting sequences (M) for post-transcriptional silencing in liver and Dorsal Root Ganglia (DRG) to avoid toxicity



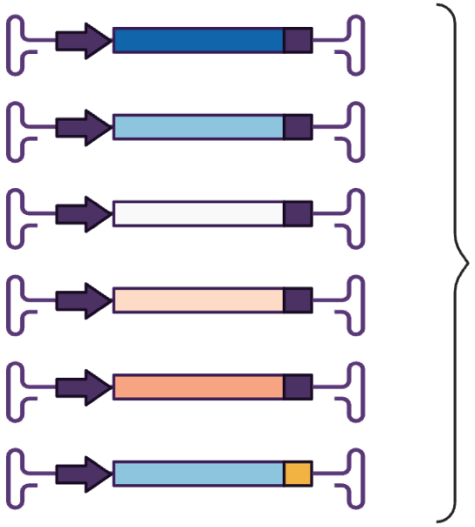


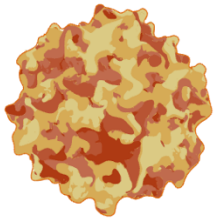
Scale = 200 µm

Hordeaux J *et al*. *Science Translational Medicine*, 2020.

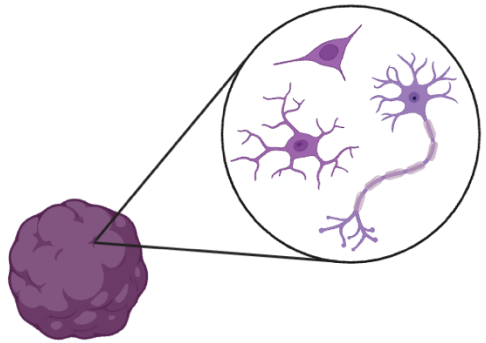
Selection of the therapeutic cassette

AAV-CTNNB1 cassettes





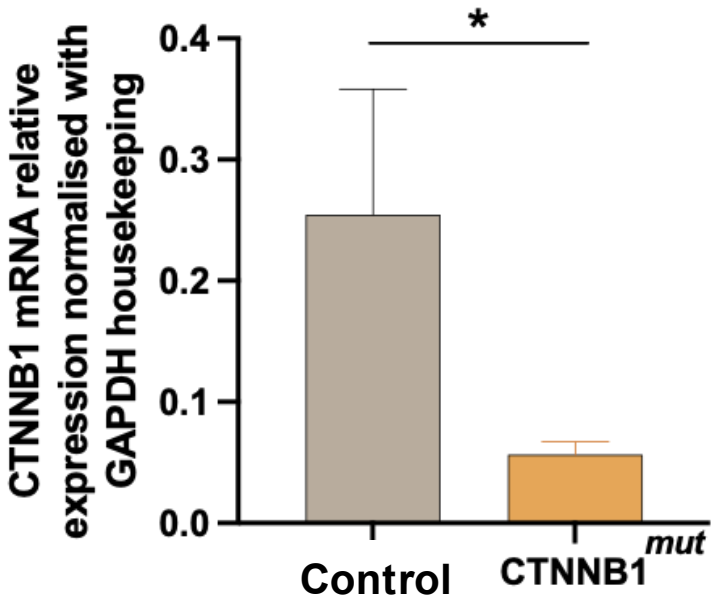
AAV vector



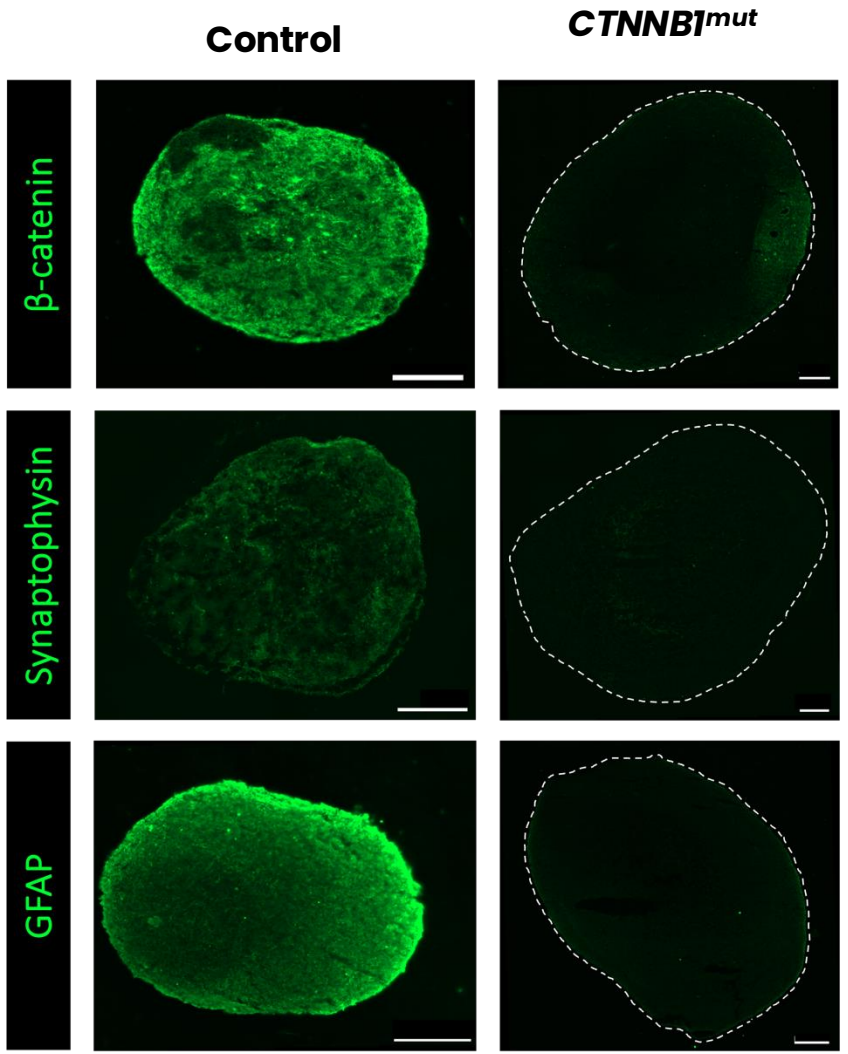
Patient-derived cortical brain organoids

Patient-derived brain organoids

β-catenin expression and function affected in patient-derived organoids



Unpaired *t* test, $p = 0.035$

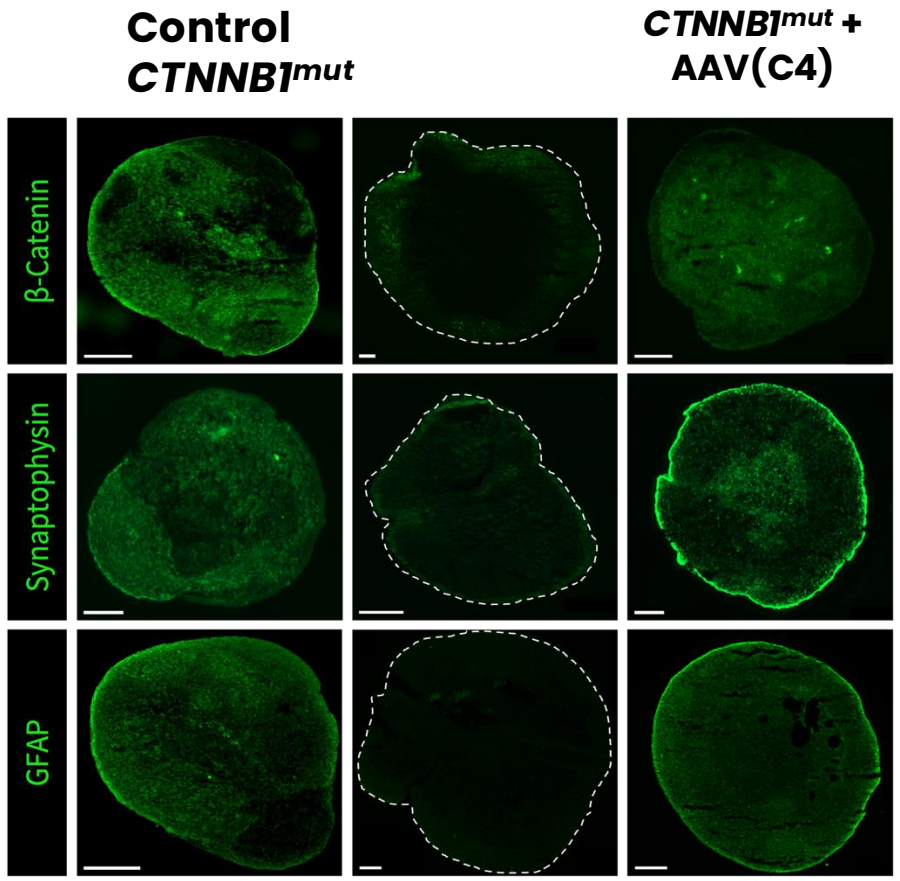


Yang *et al*. PNAS, 2011

Scale = 200 µm

GFAP: Glial Fibrillary Acidic Protein

Efficacy evaluation in patient-derived organoids



GFAP: Glial Fibrillary Acidic Protein

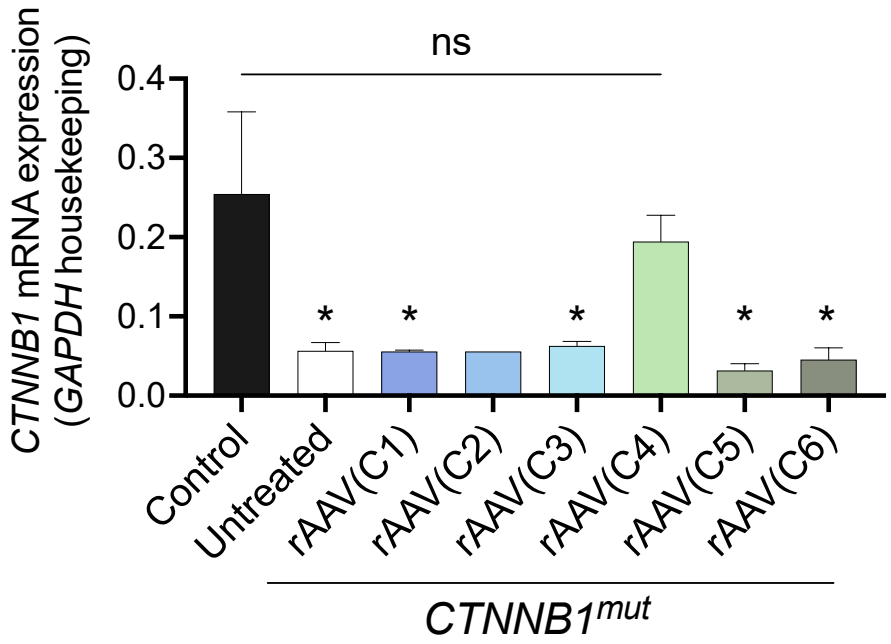
✓Recovery of β-catenin protein **expression** after treatment with AAV-CTNNB1(C4)

✓Recovery of β-catenin **function** in:

- Synaptic activity (synaptophysin)
- Astrocyte activation (GFAP)

Efficacy evaluation patient-derived organoids

- ✓ Recovery of β-catenin **mRNA expression** after treatment with AAV-CTNNB1(C4)



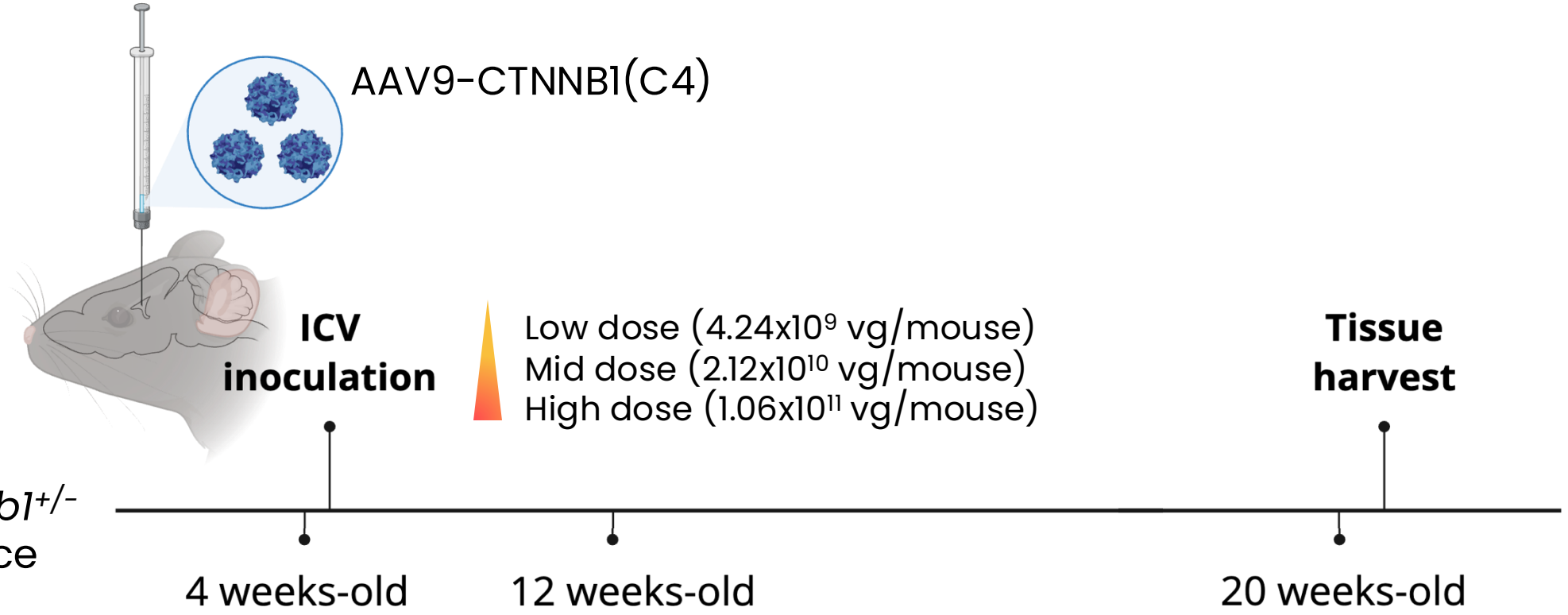
One-way ANOVA, p = 0.0122

- ✓ RNA-sequencing

Treated *vs.* Control organoids

- Oncogenic pathways
- **Wnt signaling pathway**

Efficacy evaluation in mice



Behavioural tests to assess:

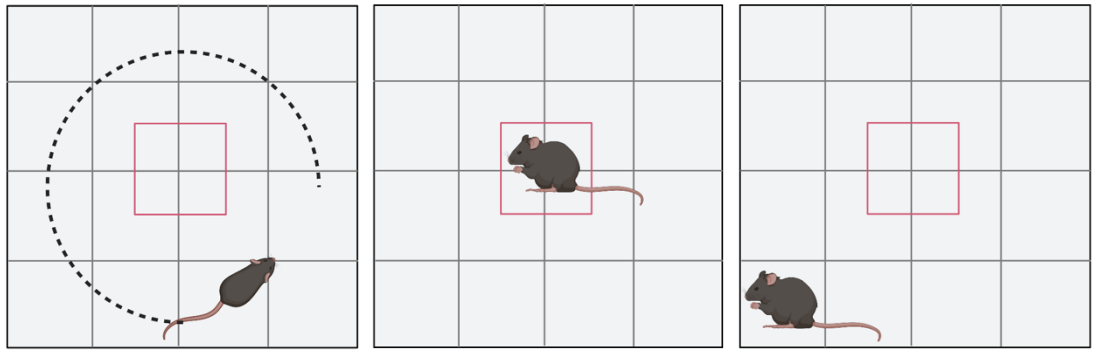
- **Motor function and coordination**
- **Anxiety-like behaviour**

ICV: Intracerebroventricular

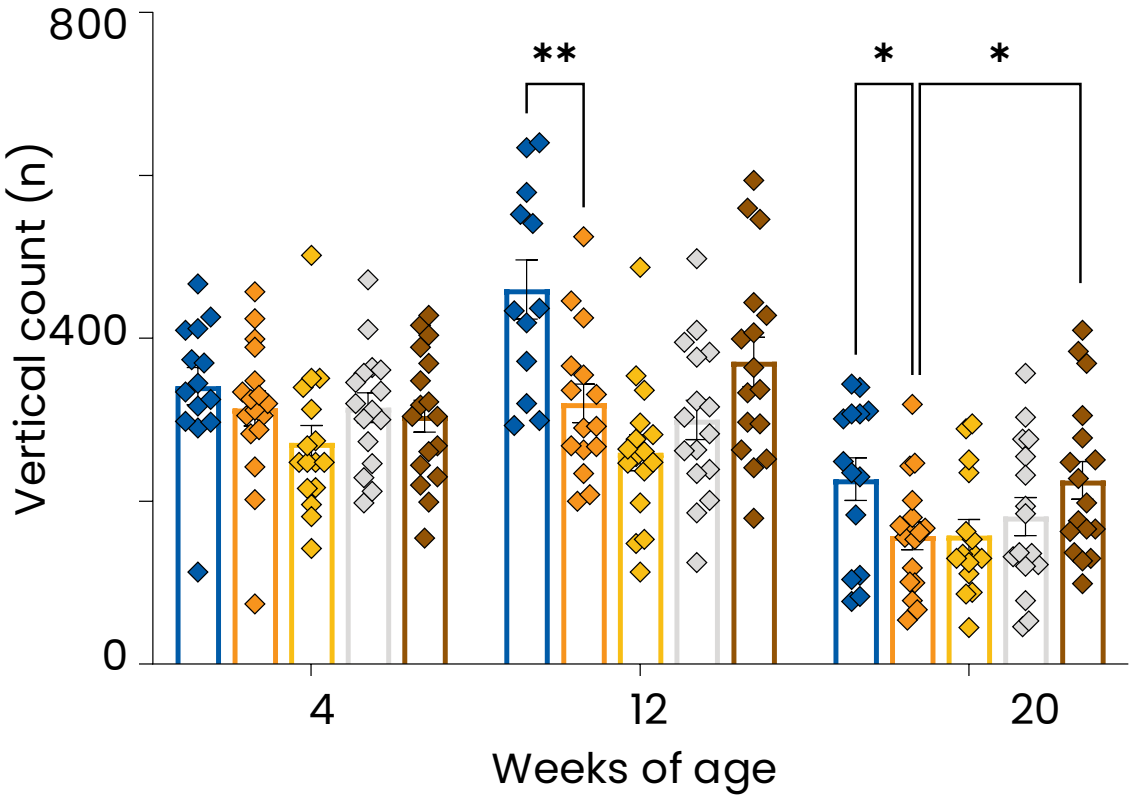
Efficacy evaluation in mice

✓ **Rescue of anxiety-like behaviour**

Open field
Rearing frequency



- Control
- *Ctnnb1*$^{+/-}$
- *Ctnnb1*$^{+/-}$ AAV9-CTNNB1(C4) Low
- *Ctnnb1*$^{+/-}$ AAV9-CTNNB1(C4) Mid
- *Ctnnb1*$^{+/-}$ AAV9-CTNNB1(C4) High



Efficacy evaluation in mice

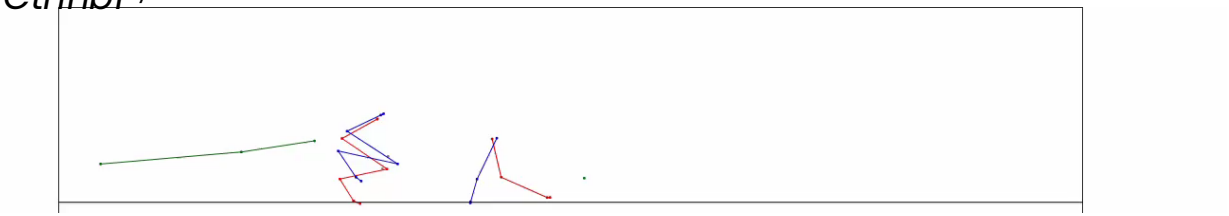
✓ Rescue of locomotor function

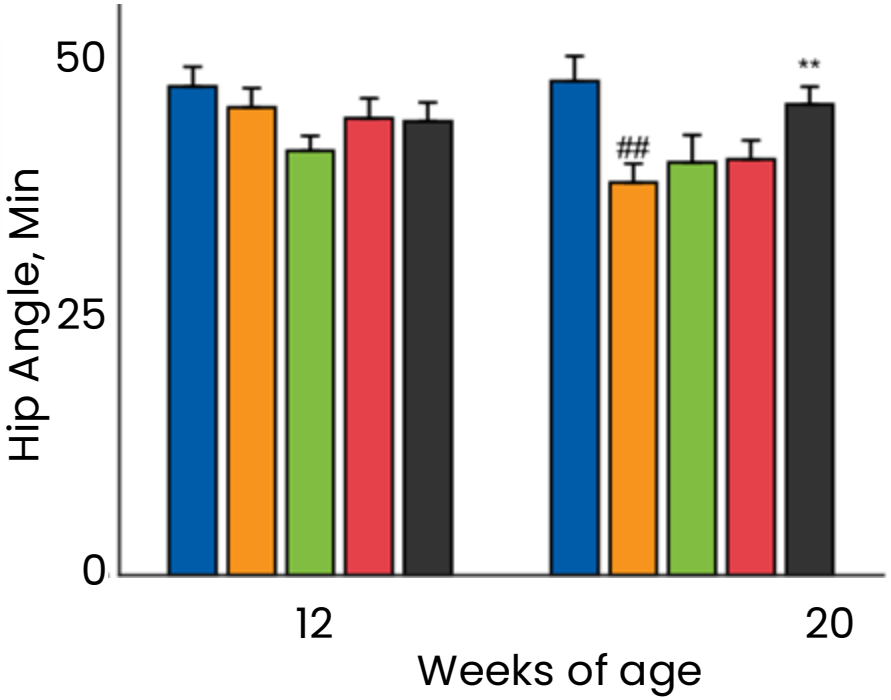
Kinematic gait analysis

Hip angle

Control

Ctnnb1$^{+/-}$





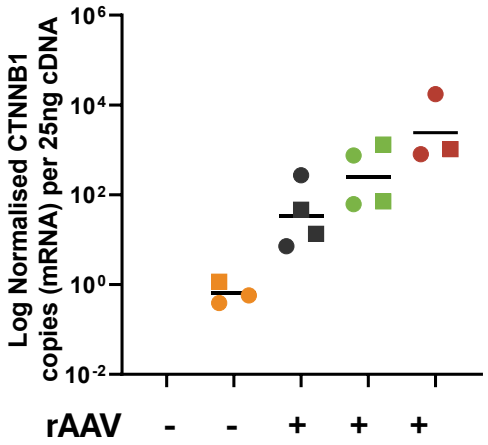
- Control
- *Ctnnb1*$^{+/-}$
- *Ctnnb1*$^{+/-}$ AAV9-CTNNB1(C4) Low
- *Ctnnb1*$^{+/-}$ AAV9-CTNNB1(C4) Mid
- *Ctnnb1*$^{+/-}$ AAV9-CTNNB1(C4) High

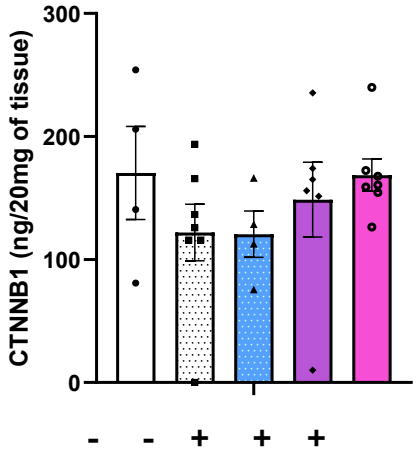
Efficacy evaluation in mice

Dose-dependent increase in mRNA and protein expression across all regions of the brain tested (hypothalamus, thalamus, hippocampus, subcortex, cerebellum)

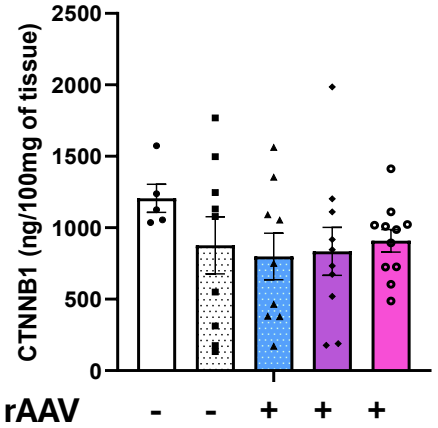
Silencing of transgene expression in the liver, mediated by the miRNA targeting sequences included in the cassette

Hypothalamus





Liver



Control
Ctnnb1$^{+/-}$
Ctnnb1$^{+/-}$ AAV9-CTNNB1(C4) Low
Ctnnb1$^{+/-}$ AAV9-CTNNB1(C4) Mid
Ctnnb1$^{+/-}$ AAV9-CTNNB1(C4) High

CTNNB1^{mut} = Disease model
- = Untreated
+ = Treated

AAV-mediated gene therapy for CTNNB1 Syndrome

CTNNB1 FOUNDATION
Bringing hope to children

EFFICACY | **BIODISTRIBUTION AND TOXICOLOGY**

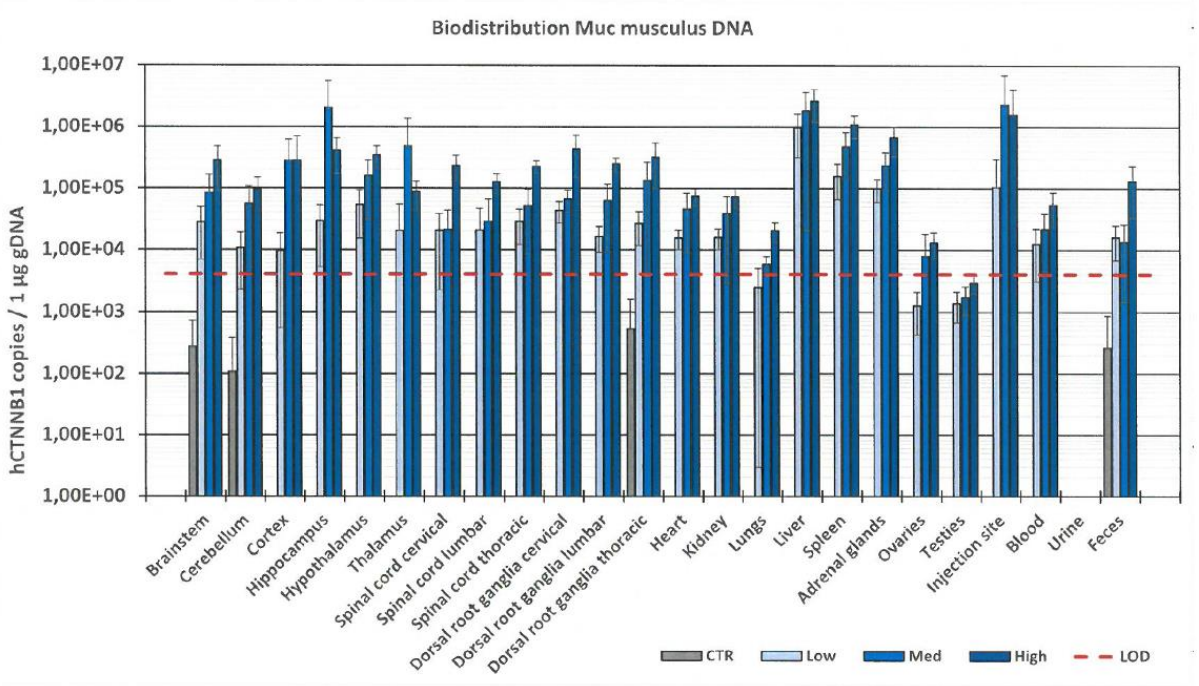
Non-GLP 5-month	Non-GLP 5-month	Non-GLP 8-month	Non-GLP 1-month	GLP 3-month	Non-GLP 3-month
					
Organoids	*Ctnnb1*$^{+/-}$	C57BL/6	C57BL/6	C57BL/6	Cynomolgus macaque
CHILDREN'S MEDICAL RESEARCH INSTITUTE	charles river	CHILDREN'S MEDICAL RESEARCH INSTITUTE	Czech Centre for Phenogenomics	Czech Centre for Phenogenomics	Cima Universidad de Navarra

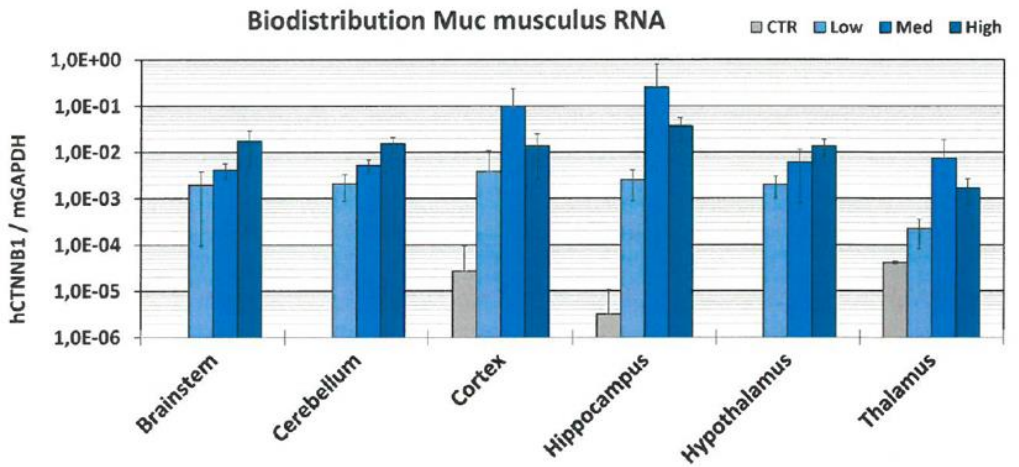
Biodistributon studies

C57BL/6

Cynomolgus macaque

Study	Design	Nominal Dose and route (vg/mouse)	Model group size (N)
PRE-2024-033	28-day non-GLP biodistribution study in juvenile mice	ICV for all animals Low: 5.00E+10 Mid: 1.00E+11 High: 3.35E+11	C57BL/6NCr mice, 48 total (12/group)
GLP-2024-03	90-day GLP toxicity and biodistribution study in juvenile mice	ICV for all animals Low: 5.00E+10 Mid: 1.00E+11 High: 3.35E+11	C57BL/6NCr mice, 48 for biodistribution arm (12/group)
NHP-2024-01	90-day non-GLP biodistribution study in cynomolgus monkeys	ICV and ICM Human dose: 3.15E+13 vg/NHP High dose: 7.85E+13 vg/NHP	Macaca fascicularis, 6 total (4M + 2F)





Toxicology studies

CHILDREN'S MEDICAL RESEARCH INSTITUTE

Czech Centre for Phenogenomics

Cima Universidad de Navarra

Non-GLP 8-month

- 2 different doses tested, GFP and AAV-CTNNB1 + 1 untreated control in WT P28 days old mice
- No significant changes in behavior and physical wellness were observed
- Blood analysis showed no significant differences in any of the groups
- Cortical thickening in all groups, more prevalent in GFP high dose females
- Overall, the study demonstrated that AAV9-CTNNB1(C4) did not induce any adverse biochemical or histopathological effects in wild type mice.

GLP 3-month

- 3 different doses tested + 1 ICV empty treated control
- Lesions (inflammation, necrosis, edema) mostly localized to injection site
- Inflammatory cell infiltration in all groups, more prevalent in high dose group
- No systematic peripheral organ toxicity
- Blood analysis confirmed no treatment-related systematic toxicity
- Overall, ICV AAV9-CTNNB1 delivery well tolerated in juvenile mice up to 3.35E+11 vg/mice (HED 1.2E+15 vg/mice)

GLP 3-month

- 2 different doses tested, bilateral ICV and ICM, no control (used historical control)
- No clinical signs or abnormal behavior observed
- Neurological exams normal in all dose groups
- All brain findings were minimal and within background incidence
- No DRG abnormalities in animals where DRG was available
- Peripheral organ findings were sporadic and consistent with normal background

URBAGEN demonstrates a favourable safety profile in toxicology testings

→ Supports further development toward clinical testing

GAIN-CTNNB1: A Phase I/II open-label, single dose clinical trial to evaluate the safety, tolerability, and preliminary efficacy of intracerebroventricular administration of an AAV9-based gene replacement therapy in paediatric patients with CTNNB1 neurodevelopmental syndrome.

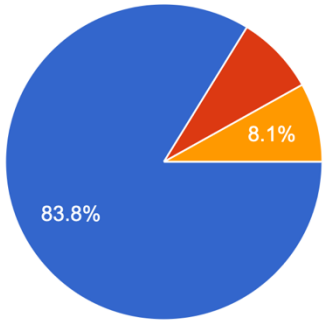
- **Study Type:** Phase I/II, open-label, single-dose
- **Population:** Paediatric patients (2–12 years) with CTNNB1 loss-of-function neurodevelopmental syndrome
- **Primary objective**: Evaluate the safety and tolerability of bilateral intracerebroventricular (ICV) delivery of AAV9-CTNNB1
- **Secondary objectives:**
  - Preliminary efficacy (clinical scales, functional assessments)
  - Biodistribution and vector shedding
  - CSF and blood biomarkers
- **Dose:** 5.0E+14 vg/patient
- **Route of administration:** Bilateral ICV infusion
- **Sample size:** Minimal 12 patients

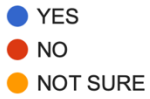
GAIN CTNNB1
gene augmentation in neurodevelopment

Interest of CTNNB1 community to participate in the GAIN-CTNNB1 clinical trial (N = 74 families)

Are you interested in your child participating in the clinical trial in Ljubljana (inclusion between 2025-2027)?

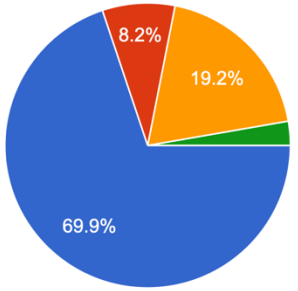
74 responses

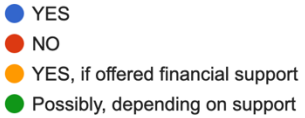




If selected, would you be able to relocate near Ljubljana for approximately 6 months and cover the related living expenses?

73 responses





Thank you!

Do you have any questions?

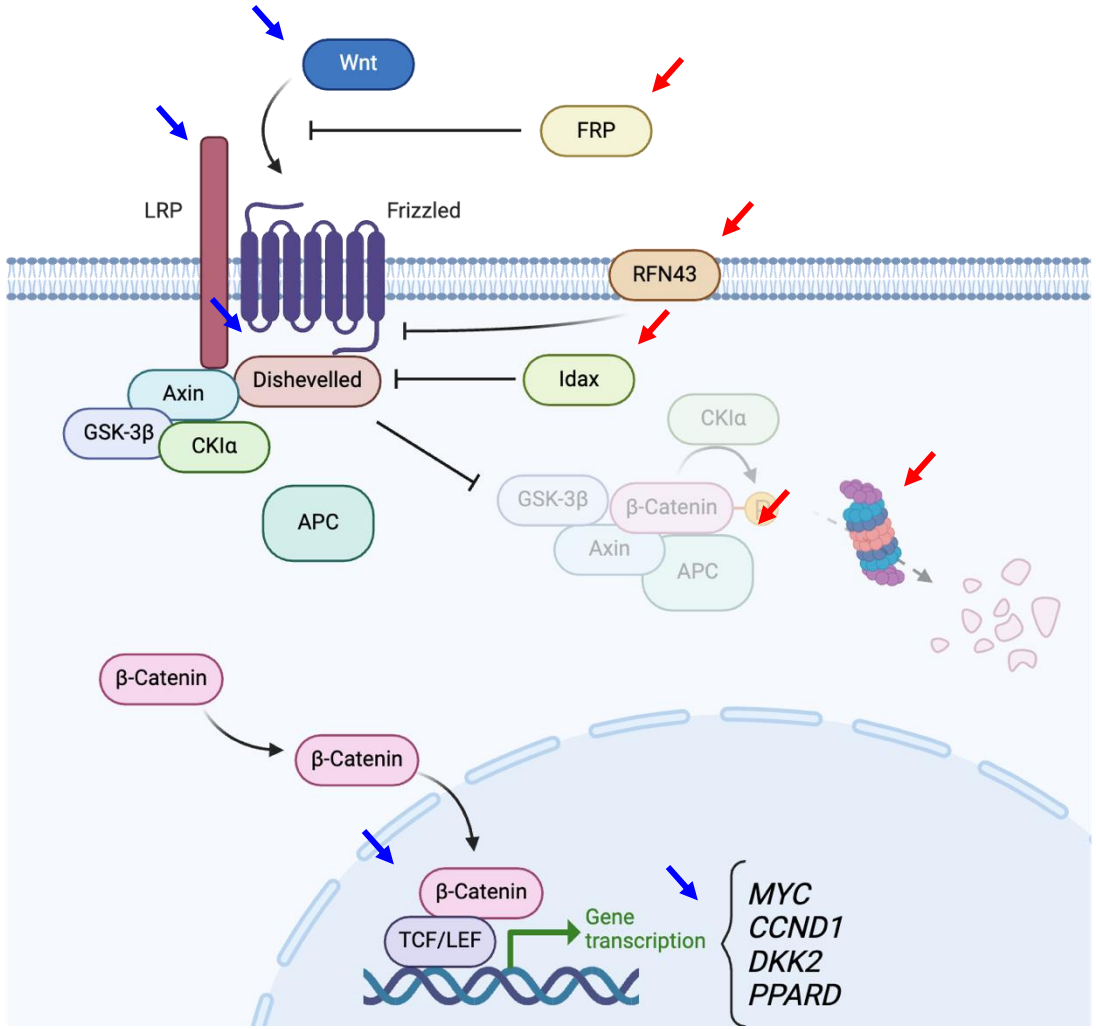
spela@ctnnb1-foundation.org

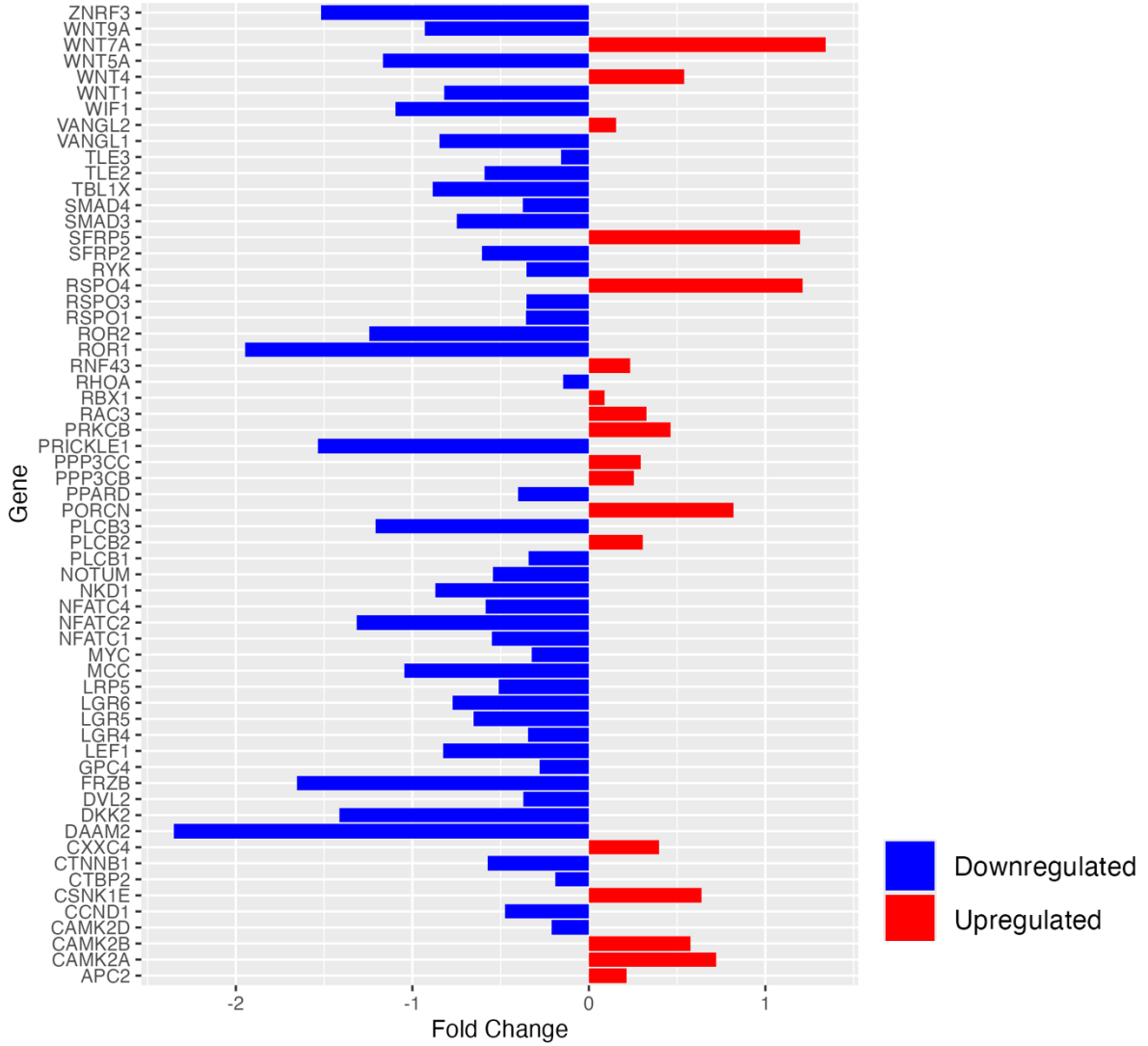
+386 31 731 269

www.ctnnb1-foundation.org

Safety evaluation in patient-derived organoids

Canonical Wnt signaling pathway

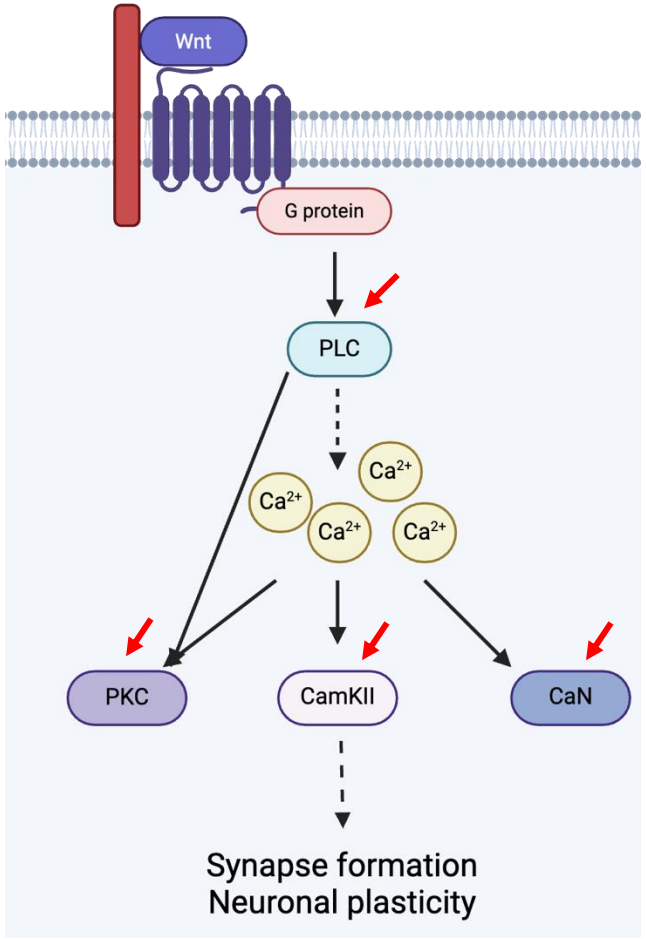


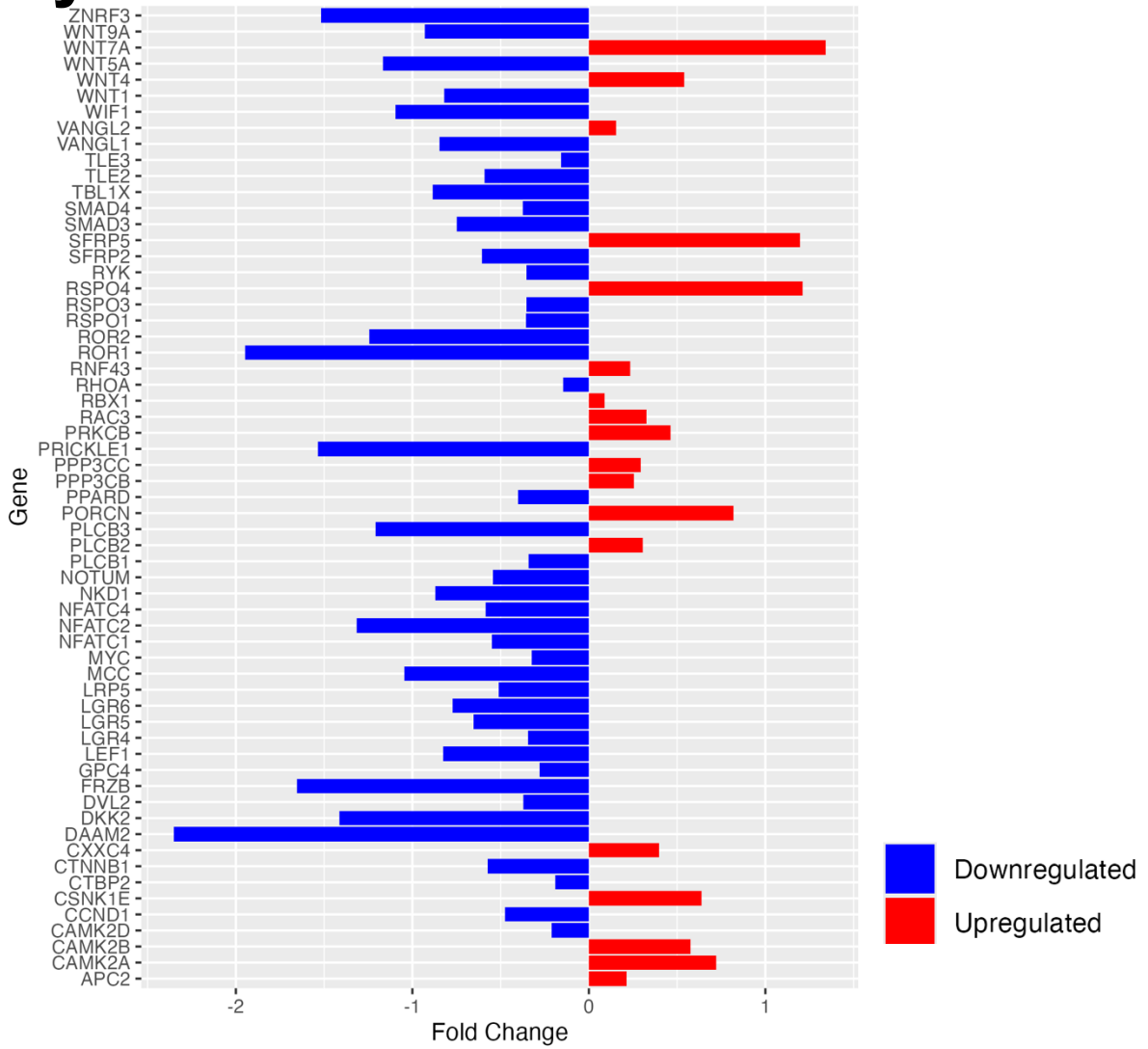


Safety evaluation in patient-derived organoids

Non-canonical Wnt signaling pathway

- Wnt/Ca^{2+} pathway





Efficacy evaluation in patient-derived organoids

Glutamatergic synapse