Supplementary Information

Supplementary Table 1: Scoring system based on clinical observation for animals in a 28- and 90-day single intracerebroventricular (ICV) dose biodistribution and toxicology studies.

Daily observation - in cage				
Symptom	**0**	**1**	**2**	**3**
Activity	Animal is active	Isolated	Huddled/Inactive OR Overactive attenuation	Completely isolated /unresponsive/ moribund Trembling/tremors/signs of pain
Body posture	Normal	Moderately hunched	Markedly hunched	Massive hunched
Coat condition	Normal	Slightly unkept, bald patches, mild piloerection,	Extensive alopecia, unkempt, piloerection, wounds, hair thinning	Whole body alopecia, very unkempt, marked piloerection, infected wounds, self-mutilation
Respiration	Normal	Increased respiratory rate	Rapid abdominal breathing	Labored, irregular breathing
Body condition	Normal	Slight deteriorated	Loss of body fat, failure to grow markedly	Significant loss of muscle mass, kachexie, runt
Weekly detailed observation – outside cage				
Body weight	Normal	Reduced growth weight	Chronic weight loss>15%	Weight loss equal to or >20%
Movement/ Gait	Normal	Reduce, slow, slight incoordination	Reluctance to move, occasional ataxia	Limb, dragging/paralysis, reduced movement on stimulation, ataxia, circling, locomotion defects
Dehydration	Normal	Skin less elastic	Mild skin tenting	Severe skin tenting, sunken eyes
Eyes, Nose / Mucosae	Normal	Wetness, dull eyes / pale or reddish	Mucoid discharge/squinty eyes / erythema	Coagulated nasal discharge, matted eyes / bluish, yellowish or blood
Faeces	Normal	Moist	Mushy	Watery or blood
Vocalization	Normal	Squeaks when palpated	Squeaks loudly when handled/palpated	Repeated abnormal vocalization

Each symptom is scored by severity from 0 to 3 points (0 point normal, 1 point mild sign of the symptom, 2 points moderate sign, 3 points severe sign of the symptom). Total score is calculated by adding up the score in each symptom; mice were euthanized with a total Clinical observation score over 5.

Supplementary Table 2: Scoring system for ophthalmological examinations in 28- and 90-day single intracerebroventricular (ICV) dose biodistribution and toxicology studies in mice.

Reaction	Grade
a) Cornea	
1) Degree of pigmentation (most pigmented area)	
no pigmentation	**0**
dispersed or diffuse areas, details of the iris clearly visible	**1**
easily recognizable transparent areas, details of the iris lightly obscured	**2**
opacifying areas, no details of the iris visible, iris size clearly distinguishable	**3**
opaque, iris details not visible	**4**
2) Affected area of the cornea	
one quarter (or less), but not zero	**0**
larger than one quarter, but less than half	**1**
larger than half, but less than a quarter | 2	**2**
larger than a quarter up to the entire area	**3**
b) Pupil	
normal	**0**
swelling above normal, lens clouding, circle around the cornea (any of these symptoms or their combination), iris reacts steadily to light (slow reaction is positive)	**1**
no reaction to light, bleeding, severe destruction (any of these symptoms)	**2**
c) Conjunctiva	
1) Redness (related to the eyelid conjunctiva and bulbar conjunctiva outside the cornea and iris)	
normal vessels	**0**
visible bleeding over normal	**1**
more diffuse, dark purple or red, individual vessels not easily recognizable	**2**
diffuse red color	**3**
2) Chemosis (swelling)	
no swelling	**0**
any swelling over normal (including mucous membrane)	**1**
visibly swelling with partial eyelid closure	**2**

swelling approximately half-closed eyelids	**3**
swelling almost completely closed eyelids	**4**
3) Size	
no swelling	**0**
any abnormal amount (not small, observed in the inner corner of normal animals)	**1**
swelling with moist eyelids and eyelash fold	**2**
swelling with moist eyelids and eyelash fold and large areas around the eye	**3**

a) Cornea: evaluation of opacity (degree) and surface area involved.

b) Iris: evaluation of pupillary light reflex, puckering, color and pericorneal swelling.

c) Conjunctiva: evaluation of the color, vascularity, chemosis, eyelid moisture, and abnormal lacrimation.

Supplementary Table 3: Reference intervals for hematology in 28- and 90-day single intracerebroventricular (ICV) dose biodistribution and toxicology studies in mice.

Parameter	Reference range	Units
WBC	2.80 - 12.60	x 10E9/L
RBC	6.92-10.85	x 10E12/L
HGB	114 - 168	g/L
HCT	0.391 - 0.540	1/100%
MCV	48.6 - 59.4	Fl
MCH	15.1 - 17.1	Pg
MCHC	278 - 323	g/L
PLT	566 - 1386	x 10^9/l
RDW - CV	0.123 - 0.188	1/100%
Neu %	0.068 - 0.421	1/100%
Neu#	0.29 - 3.55	x 10^9/L
Lym%	0.485 - 0.889	1/100%
Lym#	2.31 - 10.13	x 10^9/L
Mon%	0.020 - 0.117	1/100%
Mon#	0.11 - 1.12	x 10^9/L
Eos%	0.000 - 0.030	1/100%
Eos#	0.00 - 0.20	x 10^9/L
Bas %	0.000 - 0.002	1/100%
Bas#	0.00 - 0.03	x 10^9/L
RDW - SD	26.1 - 44.7	fl

MPV	4.9 - 5.7	fl
PDW	14.4 - 15.0	%
PCT	3.06 - 7.32	ml/L

WBC: White Blood Cell count; Neu%: Neutrophils percentage; Lym%: Lymphocytes percentages; Mon%: Monocytes percentage; Eos%: Eosinophils percentage; Bas%: Basophils percentage; Neu#: Neutrophils number; Lym#: Lymphocytes number; Mon#: Monocytes number; Eos#: Eosinophils number; Bas#: Basophils number; RBC: Red Blood Cell count; HGB: Hemoglobin Concentration; HCT: Hematocrit; MCV: Mean Cell Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Cell Hemoglobin Concentration; RDW-CV: Red Blood Cell Distribution Width Coefficient of Variation; RDW-SD: Red Blood Cell Distribution Width Standard Variation; PLT: Platelet count; MPV: Mean Platelet Volume; PDW: Platelet Distribution Width; PCT: Plateletcrit; PT: prothrombin time; APTT: activated partial thromboplastin time

Supplementary Table 4: Reference intervals for clinical biochemistry parameters in 28- and 90-day single intracerebroventricular (ICV) dose biodistribution and toxicology studies in mice.

Parameter	Reference range	Units
ALB	22.37 - 31.06	g/L
ALP	64 - 157	U/L
AST	35.00 - 125.00	U/L
ALT	15.05 - 66.73	U/L
TP	44.01 - 53.73	g/L
UREA	5.31 - 11.42	mmol/L
TRIG	0,435 - 1,641	mmol/L
CHOL	1,07 - 3,12	mmol/L
CREA	5.37 - 10.36	µmol/L
GLU	9,1 - 18,58	mmol/L
Na	134 - 154	mmol/L
K	3,68 - 6,11	mmol/L
LIH	---	---

ALB: albumin; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; TP: total protein; CREA: creatinine; GLU: glucose, TRIG: triglyceride; CHOL: cholesterol; Na: sodium; K: potassium; LIH: Lipemia/Icterus/Hemolysis

Supplementary Table 5: Histopathological grading system for mice in 28- and 90-day single intracerebroventricular (ICV) dose biodistribution and toxicology studies.

Change	Grade
No change or minimal, insignificant change.	**0**
Mild changes, with subtle or localized effects.	**1**
Moderate changes, with noticeable effects, but not severe.	**2**
Significant changes, with more pronounced effects and potential impact on organ function.	**3**
Severe changes, with widespread or extensive effects and a significant impact on the animal's health	**4**

This system assesses the extent of pathological changes, considering factors like the amount of tissue affected, the distribution of lesions (focal, multifocal, diffuse), and the complexity of the morphologic changes. Morphologic tissue changes (cysts, scar, thickened septs) are generally not graded but are routinely recorded as "present".

Supplementary Table 6: Clinical observations in the 90-day single intracerebroventricular (ICV) dose toxicology study in mice.

Dose	Number of animals with findings/total animals in the study	Humane endpoint	Clinical change observation		
Vehicle	4M/10M	0	2M-Mild alopecia; 2M-Mild eye opacity		
	4F/10F	0	3F-Mild eye opacity; 2F-alopecia		
5.00×10^{10}vg	3M/10M	0	3M-Mild alopecia; 1M-Mild increased breathing		
	1F/10F	0	1F-Mild eye opacity		
1.00×10^{11}vg	3M/10M	2	1M-euthanazition: >15% body weight drop; labored irregular breathing, apathy, tremor, incoordination	1M-euthanazition: tremor, >20% body weight drop, head swelling, piloerection, mild dehydration, apathy, incoordination	1M-Mild alopecia
	1F/10F	0	1F-Mild alopecia		
3.35×10^{11}vg	3M/10M	0	2M- Mild increased breathing; 1M-Mild alopecia		
	3F/10F	0	3F- Mild eye opacity		

Abbreviations: M = males; F = females; vg = vector genomes/mouse.

Supplementary Table 7: Gross (macroscopic) necropsy findings in the 90-day single intracerebroventricular (ICV) dose toxicology study in mice.

Dose	Number of animals with findings/total animals in the study	Macroscopic findings
Vehicle	7M/10M	2M-Mild heart enlargement; 1M-Spleen melanosis; 1M-enlarged testes and epididymis; 2M-liver steatosis; 1M-Alopecia
	5F/10F	1F- Spleen melanosis; 2F-Alopecia; 1F-slight enlarged adrenal glands; 1F-mildly enlarged stomach
5.00×10^{10}vg	6M/10M	3M-Mild steatosis of liver; 1M-Mildly enlarged heart; 1M-Slightly enlarged left kidney; 1M-Mild hyperemia of adrenal gland
	6F/10F	3F- Spleen melanosis; 2F- Paler liver; 1F-alopecia
1.00×10^{11}vg	3M/10M	1M-Smaller spleen; 1M-Megacolon; 1M-Mildly enlarged heart
	2F/10F	1F- Mild alopecia; 1F-Red edge of median part of the liver
3.35×10^{11}vg	4M/10M	1M-Mild alopecia; 2M-Spleen melanosis; 1M-Mild hyperemia of epididymis
	3F/10F	2F-Spleen melanosis; 1F-smaller liver; 1F-Eye cataract

Abbreviations: M = males; F = females; vg = vector genomes/mouse.

Supplementary Table 8: Histopathological findings (Grade 0-4) in a 90-days single Intracerebroventricular (ICV) dose (vehicle and 3.35×10^{11}vg dose group) toxicity study in mice

Organ	Pathology	Dose	No. of animals with findings	Grade (No. of animals)			
				1	2	3	4
Eyes	Retinal dysplasia	vehicle	1M		1		
		3.35×10^{11}vg	2M	2			
	Corneal cyst	vehicle	1M, 1F	/			
Pituitary gland	Cyst, pars distalis	vehicle	2M	/			
		3.35×10^{11}vg	1F, 1M				
	Cyst, pars nervosa	vehicle	1M	/			
Adrenal glands	Subcapsular hyperplasia	vehicle	6F	5	1		
		3.35×10^{11}vg	1M, 7F	7	1		

Esophagus	Hyperkeratosis	3.35×10^{11}vg	1M		1		
Duodenum	Villi degeneration	vehicle	1F	1			
		3.35×10^{11}vg	1M, 2F	3			
Ileum	Villi degeneration	vehicle	2M, 1F	3			
		3.35×10^{11}vg	2M, 2F	4			
Jejunum	Villi degeneration	3.35×10^{11}vg	1M, 2F	3			
Liver	Focal inflammation	vehicle	2M, 3F	5			
		3.35×10^{11}vg	5M, 3F	8			
Pancreas	Acinar degeneration	3.35×10^{11}vg	2M, 3F	5			
Mesenterial lymph nodes	Cellular infiltration-histiocytosis	vehicle	1F		1		
		3.35×10^{11}vg	3M, 3F	2	1		
Spleen	Extramedullary hemopoiesis	vehicle	4M, 4F	/			
		3.35×10^{11}vg	2M, 3F	/			
Thymus	Cyst	vehicle	8M, 3F	/			
		3.35×10^{11}vg	2M, 5F	/			
	Lymphoid atrophy	vehicle	2F	1	1		
		3.35×10^{11}vg	2F	1	1		
Heart	Cardiomyocyte vacuolation	3.35×10^{11}vg	2M	2			
	Focal necrosis	3.35×10^{11}vg	1M	1			
Kidney	Renal tubule degeneration	3.35×10^{11}vg	1M		1		
Testis	Germ cells exfoliation	vehicle	4M	4			
		3.35×10^{11}vg	5M	5			
	Germ cells vacuolation	3.35×10^{11}vg	9M	9			
Epididymis	Exfoliated germ cells	vehicle	2M	2			
		3.35×10^{11}vg	5M	5			
Seminal vesicle	Hypertrophy	vehicle	2M	2			
		3.35×10^{11}vg	2M	2			
Prostate gland	Acini dilatation	3.35×10^{11}vg	1M	1			
	Secretory alteration	3.35×10^{11}vg	1M	1			
Spinal cord - Cervical	Demyelination	vehicle	3M, 2F	2	1		
		3.35×10^{11}vg	4M, 2F		3	3	
	Perivascular edema	vehicle	1F	1			
		3.35×10^{11}vg	1M		1		
Spinal cord - Thoracic	Demyelination	vehicle	3M, 3F	2	1	3	
		3.35×10^{11}vg	5M, 5F	4	2	4	
	Cyst	vehicle	1M	/			
	Demyelination	vehicle	3M, 2F	3	2		

Spinal cord - Lumbar		3.35×10^{11}vg	5M, 2F	2	5		
Femur	Bone marrow, hypocellularity	vehicle	1F	1			
		3.35×10^{11}vg	3F	2	1		
	Bone marrow, hypercellularity	vehicle	2F	1	1		
		3.35×10^{11}vg	1F	1			

Abbreviations: M = males; F = females; vg = vector genomes/mouse: /-grade not given; change was observed

Supplementary Table 9: Histopathological findings (Grades 0-4) of animals reaching humane endpoints in a 90-day single intracerebroventricular (ICV) dose toxicology study; two male mice in dose group 1×10^{11} vg/mouse.

Organ	Pathology	Number of animals with findings/ Total number of animals	Grade	
			Mouse1	Mouse2
Adrenal glands	Subcapsular hyperplasia	1/2	1	0
Esophagus	Hyperkeratosis	2/2	1	1
Stomach	Ulcer	2/2	1	2
	Inflammatory infiltration	2/2	1	2
Duodenum	Epithelial hyperplasia	2/2	1	1
Ileum	Villi degeneration	2/2	1	1
	Epithelial hyperplasia	2/2	1	1
	Inflammatory infiltration in submucosa and mesentery	2/2	2	2
	Necrosis	2/2	2	2
Jejunum	Villi degeneration	2/2	1	1
	Epithelial hyperplasia	2/2	1	1
	Inflammatory infiltration in submucosa and mesentery	2/2	2	2
Cecum	Inflammatory infiltration in submucosa and mesentery	2/2	1	1
	Crypt degeneration	2/2	1	1
	Ulcer	1/2	0	2
Colon	Epithelial hyperplasia	2/2	1	1
	Mononuclear infiltration	2/2	1	1
Liver	Atrophy	2/2	2	2
Pancreas	Acinar vacuolation	1/2	2	0
	Necrosis	1/2	2	0
Mesenterial lymph nodes	Necrosis	2/2	2	2
Spleen	Atrophy	2/2	3	3

	Extramedullary hemopoiesis	2/2	/	/
Thymus	Necrosis	2/2	2	2
	Lymphoid atrophy	1/2	2	0
Heart	Cardiomyocyte vacuolation	2/2	1	1
	Multifocal necrosis	2/2	2	2
Lungs	Thickened septs	2/2	/	/
Kidney	Renal tubule degeneration	2/2	2	2
Testis	Germ cells exfoliation	2/2	1	1
	Germ cells vacuolation	2/2	2	2
Epididymis	Exfoliated germ cells	2/2	1	1
Seminal vesicle	Hypertrophy	2/2	1	1
Spinal cord - Cervical	Hemorrhage	2/2	/	/
Spinal cord - Thoracic	Demyelination	1/2	1	0
Femur	Bone marrow, hypocellularity	2/2	2	2

Abbreviations: vg = vector genomes/mouse; / = grade not given, change was observed

Supplementary Table 10: Clinical observations in 90- and 180-day single intracerebroventricular (ICV) dose biodistribution studies in mice.

Dose	Number of animals with findings/total animals in the study	Humane endpoint	Clinical change observation
Vehicle	1M/6M	0	Mild alopecia
	0F/6F	0	/
5.00×10^{10}vg	1M/6M	0	Mild alopecia
	2F/6F	0	1F-Barbering; 2F-Mild eye opacity
1.00×10^{11}vg	2M/6M	0	2M-Mild eye opacity
	0F/6F	0	/
3.35×10^{11}vg	1M/6M	0	Mild alopecia
	2F/6F	0	2F-Mild eye opacity

Abbreviations: M = males; F = females; vg = vector genomes/mouse; / = grade not given, change was observed

Supplementary Table 11: Gross (macroscopic) findings at necropsy in the 90-day single intracerebroventricular (ICV) dose biodistribution study in mice.

Dose	Number of animals with findings/total animals in the study	Macroscopic findings
Vehicle	1M/3M	Mildly enlarged heart
	1F/3F	Mildly enlarged spleen, spleen melanosis
5.00×10^{10}vg	1M/3M	Mild alopecia
	3F/3F	1F- Mildly enlarged spleen, spleen melanosis; 2F- Mildly enlarged spleen; 3F-alopecia
1.00×10^{11}vg	1M/3M	Mild eye cataract
	1F/3F	1F- Mildly enlarged spleen, spleen melanosis
3.35×10^{11}vg	1M/3M	Mildly enlarged spleen
	1F/3F	Mild eye opacity, spleen melanosis

Abbreviations: M = males; F = females; vg = vector genomes/mouse

Supplementary Table 12: Gross (macroscopic) findings at necropsy in the 180-day single intracerebroventricular (ICV) dose biodistribution study in mice.

Dose	Number of animals with findings/total animals in the study	Macroscopic findings
Vehicle	1M/3M	Mildly enlarged spleen
	0F/3F	/
5.00×10^{10}vg	1M/3M	Spleen melanosis, alopecia
	0F/3F	/
1.00×10^{11}vg	0M/3M	/
	0F/3F	/
3.35×10^{11}vg	1M/3M	Fatty liver, enlarged seminal vesicles
	1F/3F	Mildly enlarged spleen, uterus, left eye cataract

Abbreviations: M = males; F = females; vg = vector genomes/mouse; / = grade not given, change was observed

Supplementary Table 13: Histopathological grading system for *Macaca fascicularis* in the 90-day single-dose intracerebroventricular (ICV) biodistribution and toxicology study.

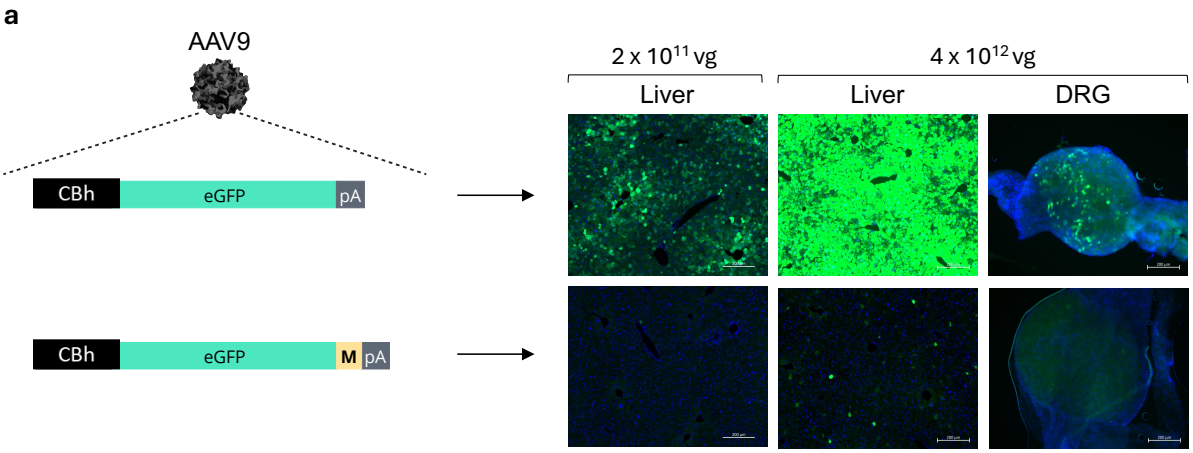
Change	Grade
No findings	**0**
Minimal/very few/very small changes	**1**
Slight/few/small changes	**2**

This system assesses the extent of pathological changes, considering factors the proportion of tissue affected, the lesion distribution (focal, multifocal, diffuse), and the complexity of the morphologic alterations.

Supplementary Table 14: Histopathology scores of *Macaca fascicularis* in the 90-day single-dose intracerebroventricular (ICV) biodistribution and toxicology study.

Animal	Dose	Region	Change	Grade
1	**ICV 3.15×10^{13}vg**	Frontal pole	Mononuclear cells focal, lateral fissure	1
		Anterior commissure	Brownish pigment in optical chiasm	1
		Lungs	Brownish pigment	1
		Liver	Inflammatory cells focal	1
		Kidney	Inflammatory cells focal	1
		Spleen	hemosiderin	1
		Thymus	Atrophy/involution	2
2	**ICV 3.15×10^{13}vg**	Anterior commisure	Mononuclear cells focal in optical chiasm	1
		Lungs	Brownish pigment	1
			Mononuclear cells focal	1
		Liver	Inflammatory cells focal	2
		Testes	Immature bilateral	
		Thymus	Single cyst	
			Atrophy/involution	2
1	**ICV 7.85×10^{13}vg**	Anterior commisure	Mononuclear cells focal	1
		Schiatic nerve	Inflammatory cells focal	1

		Lungs	Brownish pigment	1
		Testes	Immature bilateral	
		Thymus	Atrophy/involution	2
2	**ICV** **7.85×10^{13}vg**	Heart	Mononuclear cells focal	1
		Lungs	Brownish pigment	1
		Kidney	Inflammatory cells focal	1
		Testes	Immature bilateral	
		Spleen	hemosiderin	1
		Thymus	Atrophy/involution	2
1	**ICM** **3.15×10^{13}vg**	Anterior commisure	Mononuclear cells focal	1
		Lungs	Brownish pigment	1
		Kidney	Inflammatory cells focal	1
		Testes	Immature bilateral	
		Thymus	Atrophy/involution	2
2	**ICM** **3.15×10^{13}vg**	Schiatic nerve	Inflammatory cells focal	1
		Lungs	Brownish pigment	1
		Liver	Inflammatory cells focal	1
		Thymus	Atrophy/involution	2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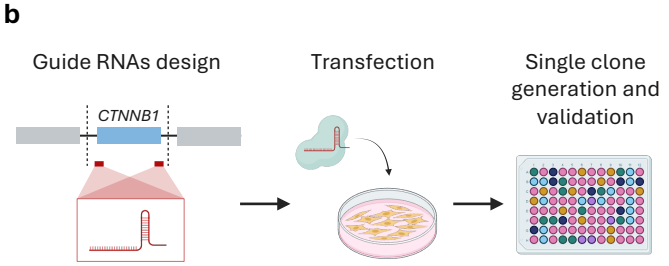
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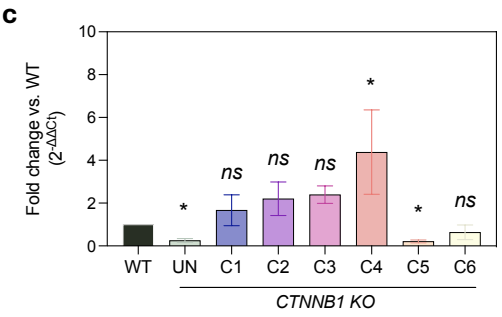
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Fold change vs. WT (2^-ΔΔCt)
WT
UN
C1
C2
C3
C4
C5
C6
CTNNB1 KO
ns
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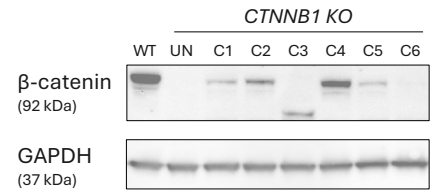
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UN
C1
C2
C3
C4
C5
C6
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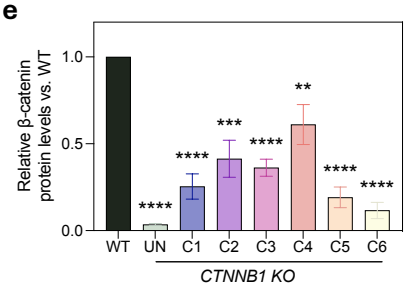
GAPDH
(37 kDa)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Relative β-catenin protein levels vs. WT
CTNNB1 KO
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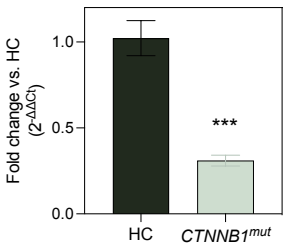
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Fold change vs. HC (2^-ΔΔCt)
HC
CTNNB1mut
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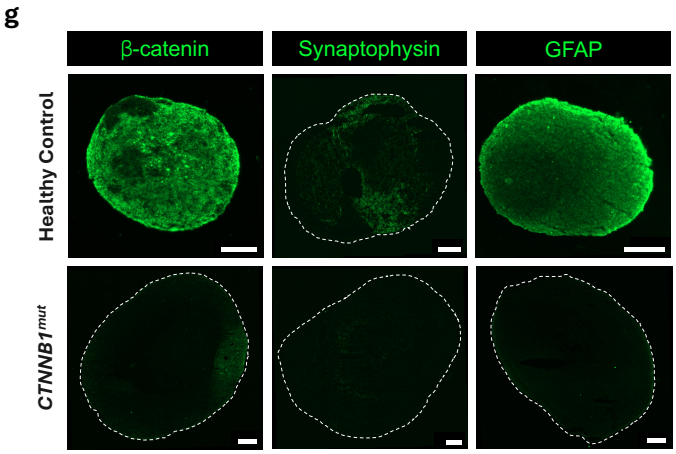
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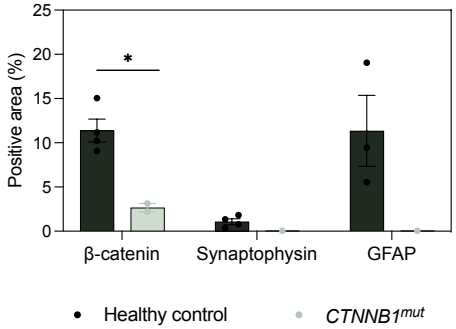
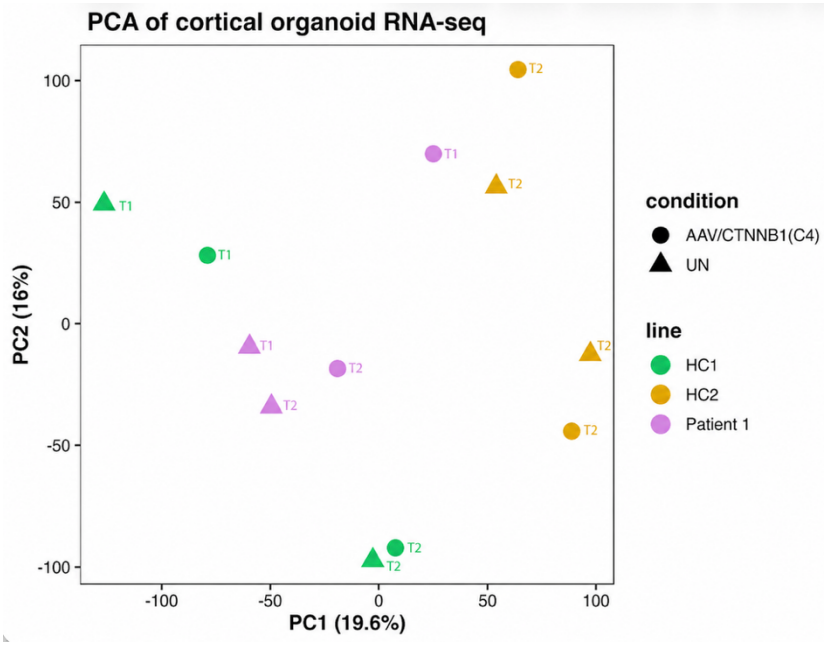
β-catenin
Synaptophysin
GFAP
Healthy Control
CTNNB1mut

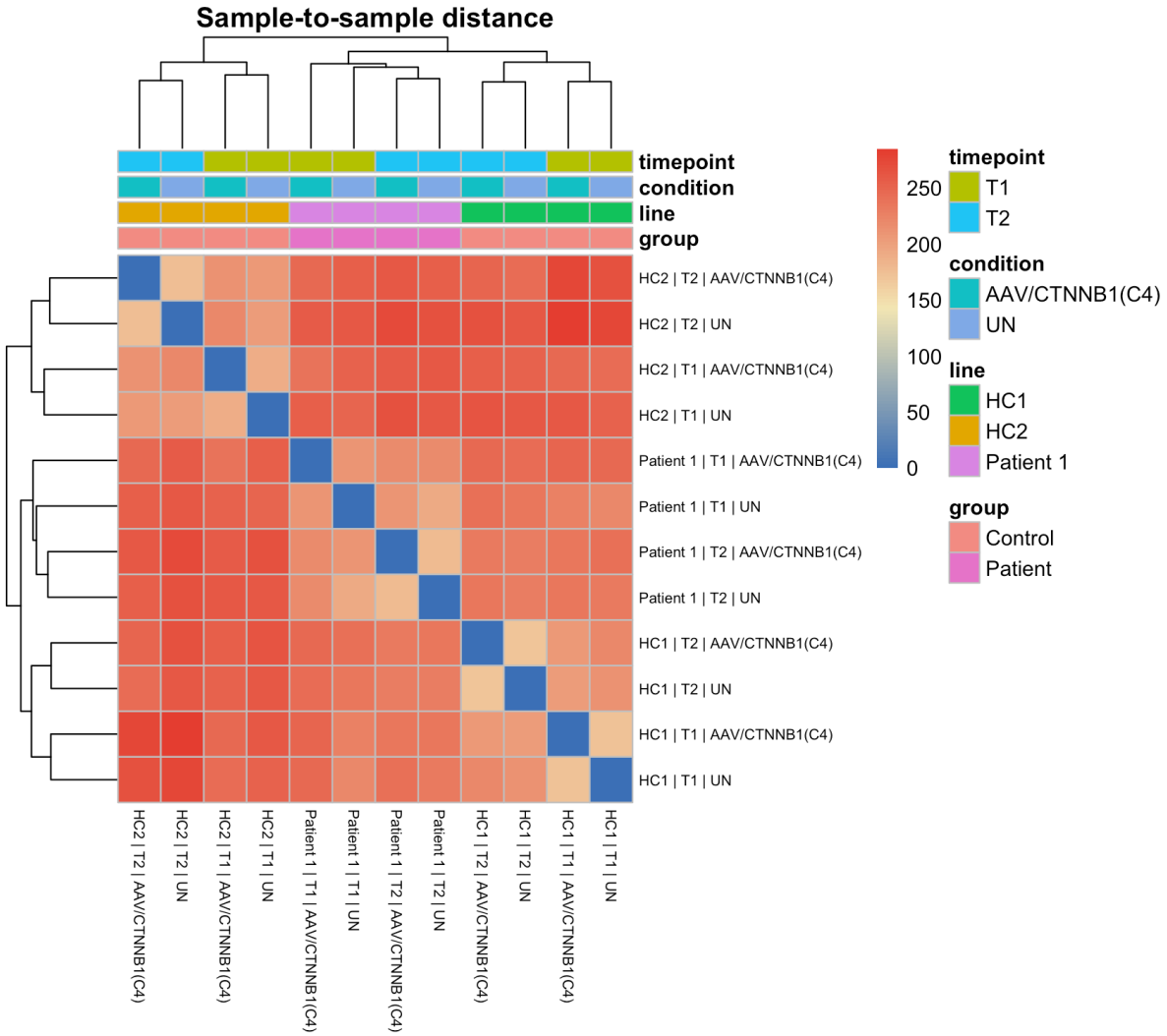

h
Positive area (%)
*
β-catenin
Synaptophysin
GFAP
Healthy control
CTNNB1mut

Supplementary Figure 1. Validation of expression cassettes and preclinical models. (**a**) *In vivo* validation of microRNA-targeting sequences (M). Humanized FRG mice (n = 2/group) received intravenous AAV9-eGFP vector (2.00×10^{11} or 4.00×10^{12} vector genomes, vg) engineered with or without microRNA-targeting sequences. Liver and dorsal root ganglia (DRG) were collected four weeks post-administration to assess eGFP expression. Enhanced Green Fluorescent Protein (eGFP) expression was controlled by the cytomegalovirus/chicken β-actin hybrid (CBh) promoter and the bovine growth hormone polyadenylation (pA) signal. Representative images are shown. Scale bar ~ 200 μm. (**b**) *CTNNB1* knockout (KO) HEK293 cells were generated by CRISPR-Cas9-mediated gene deletion and used for initial validation of the AAV-CTNNB1 constructs. (**c**) *CTNNB1* mRNA expression levels in *CTNNB1* KO HEK293 cells either untreated (UN) or transduced with AAV-CTNNB1 constructs (C1-6). Data (n = 3) are shown as fold change (mean ± SEM) relative to the wild-type (WT), calculated using the $2^{-\Delta\Delta Ct}$ (Livak) method. Statistical comparisons were performed on $-\Delta\Delta Ct$, using one-way ANOVA ($p = 0.0005$) and Fisher's Least Significant Difference (LSD) multiple comparisons test against WT group (*ns*: non-significant, * $p < 0.05$). (**d**, **e**) β-catenin protein expression levels in wild-type and *CTNNB1* KO HEK293 cells either untreated (UN) or transduced with AAV-CTNNB1 constructs (C1-6). (**d**) Western-blot images and (**e**) their β-catenin semi-quantification (n = 3 biological replicates). One-way ANOVA < 0.0001, Dunnett's multiple comparisons test (** $p = 0.0059$, *** $p = 0.0001$, **** $p < 0.0001$). (**f**) *CTNNB1* mRNA expression levels in patient-derived organoids. Data shown as fold change (mean ± SEM) relative to the healthy control (HC), calculated using the $2^{-\Delta\Delta Ct}$ (Livak) method. Statistical comparison was performed on $-\Delta\Delta Ct$, using two-tailed t-test ($p = 0.0003$). (**g**) Representative 20× immunofluorescence images of β-catenin, synaptophysin, and the Glial Fibrillary Acidic Protein (GFAP) in HC and $CTNNB1^{mut}$ organoids. Scale bar 200 μm. (**h**) Semi-quantification of immunofluorescence images. * $p < 0.05$ Unpaired *t*-test.

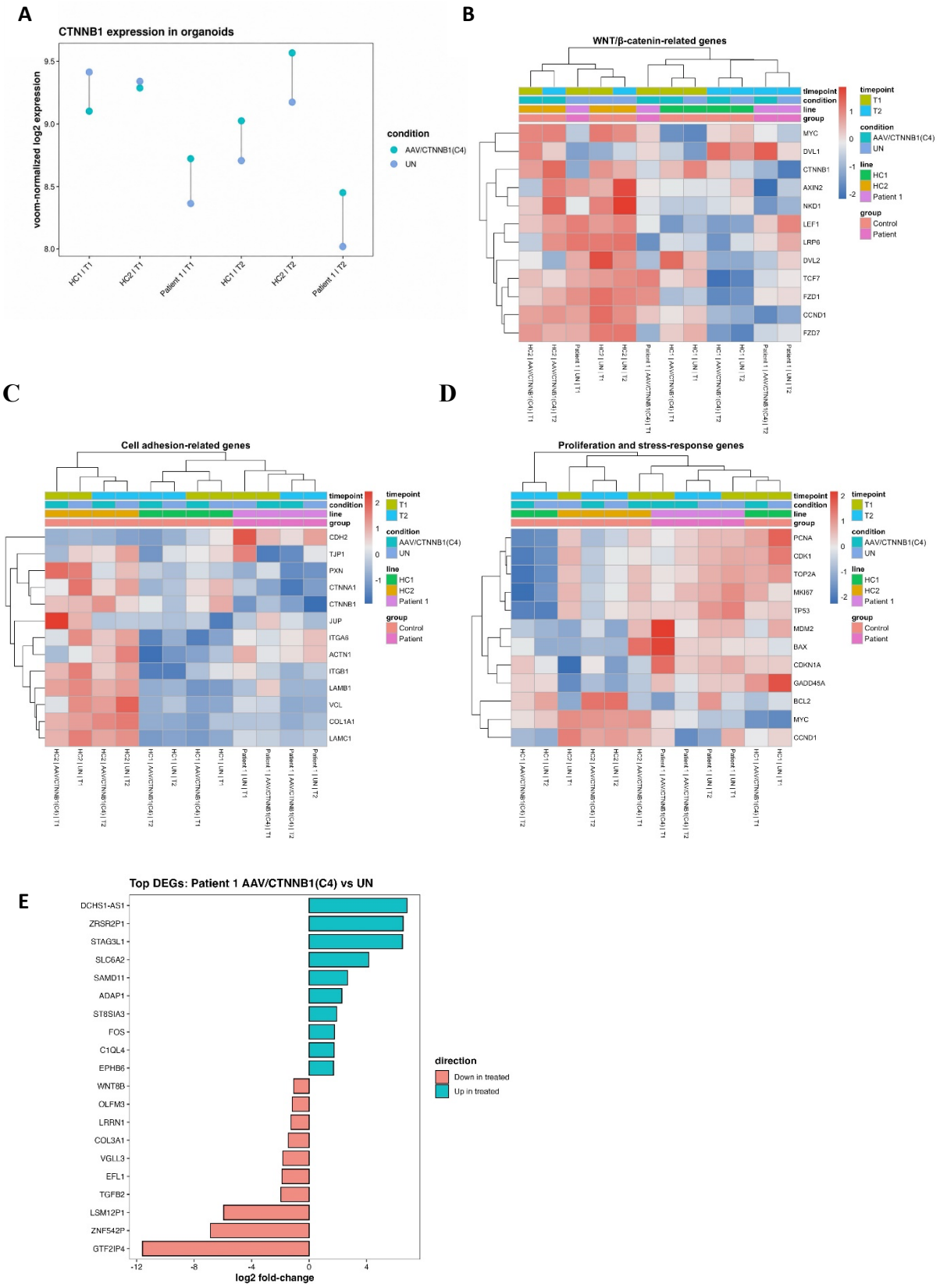
a


PCA of cortical organoid RNA-seq
PC2 (16%)
PC1 (19.6%)
100
50
0
-50
-100
-100
-50
0
50
100
T1
T2
condition
AAV/CTNNB1(C4)
UN
line
HC1
HC2
Patient 1

b

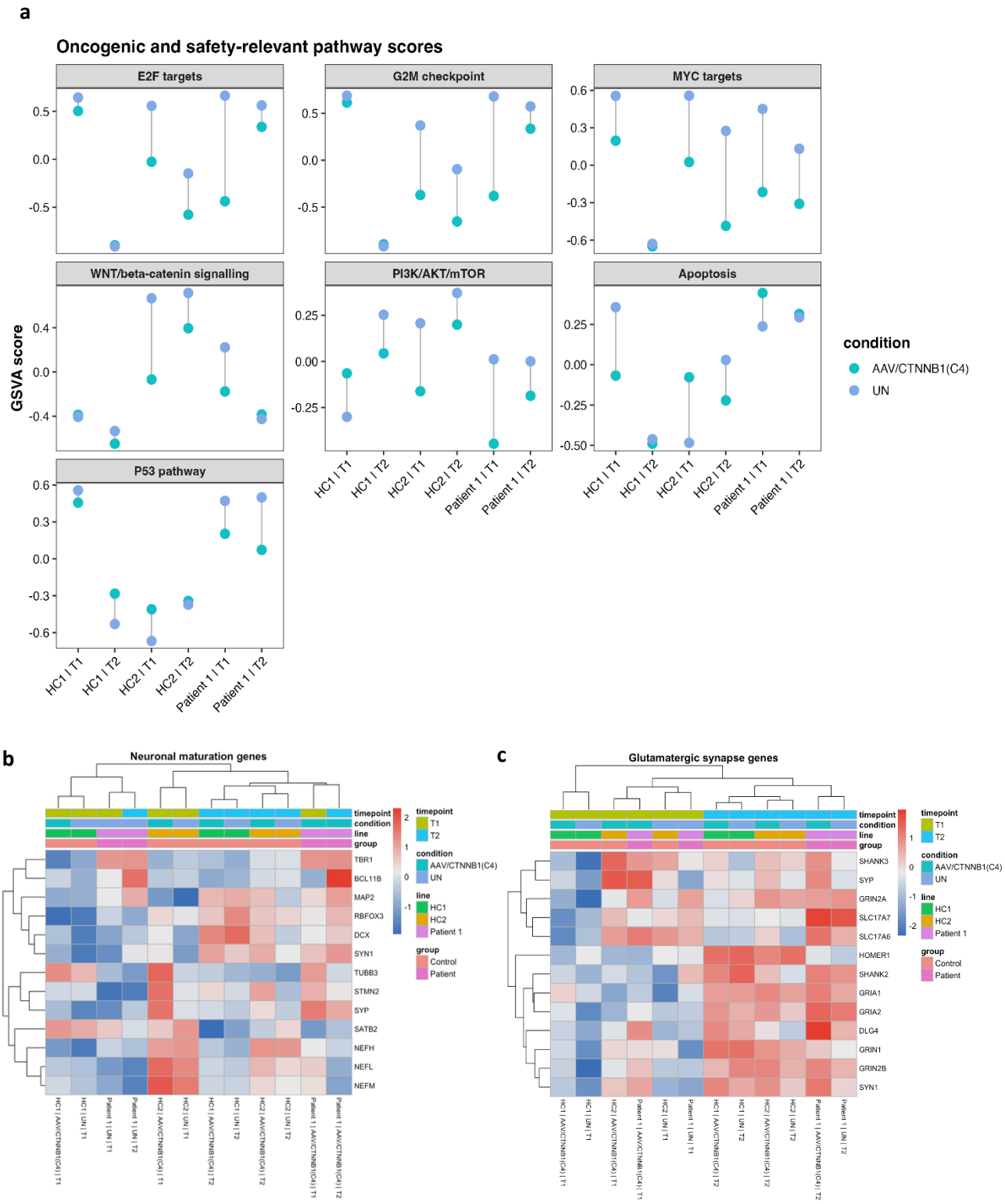

Sample-to-sample distance
timepoint
condition
line
group
HC2 | T2 | AAV/CTNNB1(C4)
HC2 | T2 | UN
HC2 | T1 | AAV/CTNNB1(C4)
HC2 | T1 | UN
Patient 1 | T1 | AAV/CTNNB1(C4)
Patient 1 | T1 | UN
Patient 1 | T2 | AAV/CTNNB1(C4)
Patient 1 | T2 | UN
HC1 | T2 | AAV/CTNNB1(C4)
HC1 | T2 | UN
HC1 | T1 | AAV/CTNNB1(C4)
HC1 | T1 | UN
250
200
150
100
50
0
timepoint
T1
T2
condition
AAV/CTNNB1(C4)
UN
line
HC1
HC2
Patient 1
group
Control
Patient

Supplementary Figure 2. Quality assessment of the cortical organoid RNA-seq dataset. **(a)** Principal component analysis (PCA) of bulk RNA-seq data from cortical organoids, including patient-derived organoids and two control lines (HC1 and HC2), under untreated (UN) and AAV-CTNNB1(C4)-treated conditions at timepoint 1 (T1) and timepoint 2 (T2). Samples are colored by line and shaped by treatment condition. **(b)** Sample-to-sample distance heatmap of the same dataset, showing hierarchical clustering based on overall transcriptomic similarity. Annotation bars indicate timepoint, treatment condition, line and group. Together, these analyses support the internal consistency and interpretability of the dataset used for downstream transcriptomic analyses.



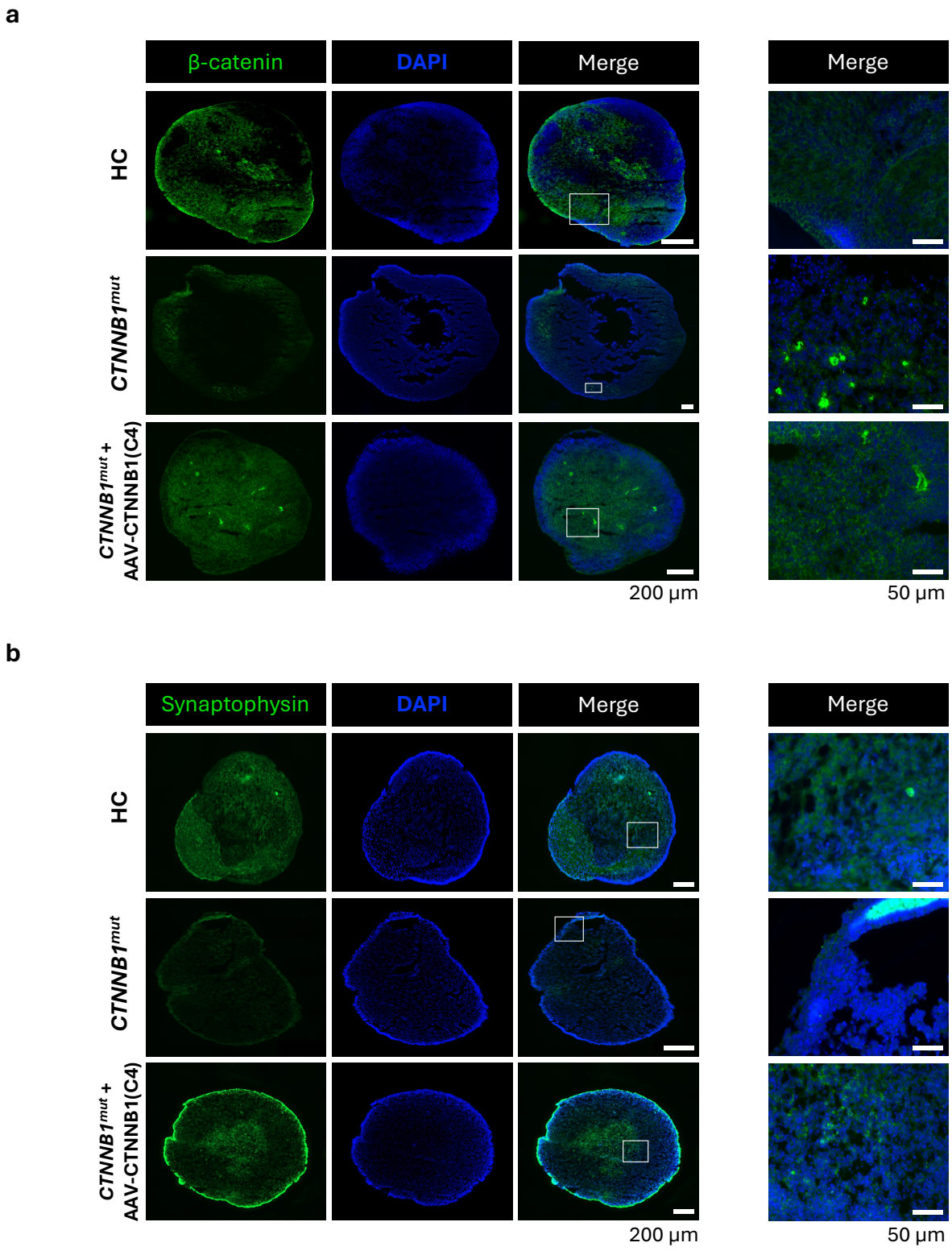
Supplementary Figure 3. Target engagement, β-catenin-related pathway changes and safety-related transcriptomic assessment in cortical organoids. Bulk RNA-seq analysis was

performed on cortical organoids derived from CTNNB1 patient and two control lines (HC1 and HC2), under untreated (UN) and AAV-CTNNB1(C4)-treated conditions at T1 and T2. **(A)** *CTNNB1* expression across control and patient organoids in treated and untreated conditions. **(B)** Heatmap of Wnt/β-catenin-related genes. **(C)** Heatmap of cell adhesion-related genes. **(D)** Heatmap of proliferation- and stress-response-related genes used as a safety-oriented transcriptional readout. Heatmaps show row-scaled expression values, with annotation bars indicating timepoint, treatment condition, line and group. **(E)** Top differentially expressed genes in treated versus untreated patient-derived organoids. Together, these analyses support target engagement and biologically coherent pathway-level effects of AAV-CTNNB1(C4) without evidence of a broad treatment-associated transcriptional safety signal in this dataset.



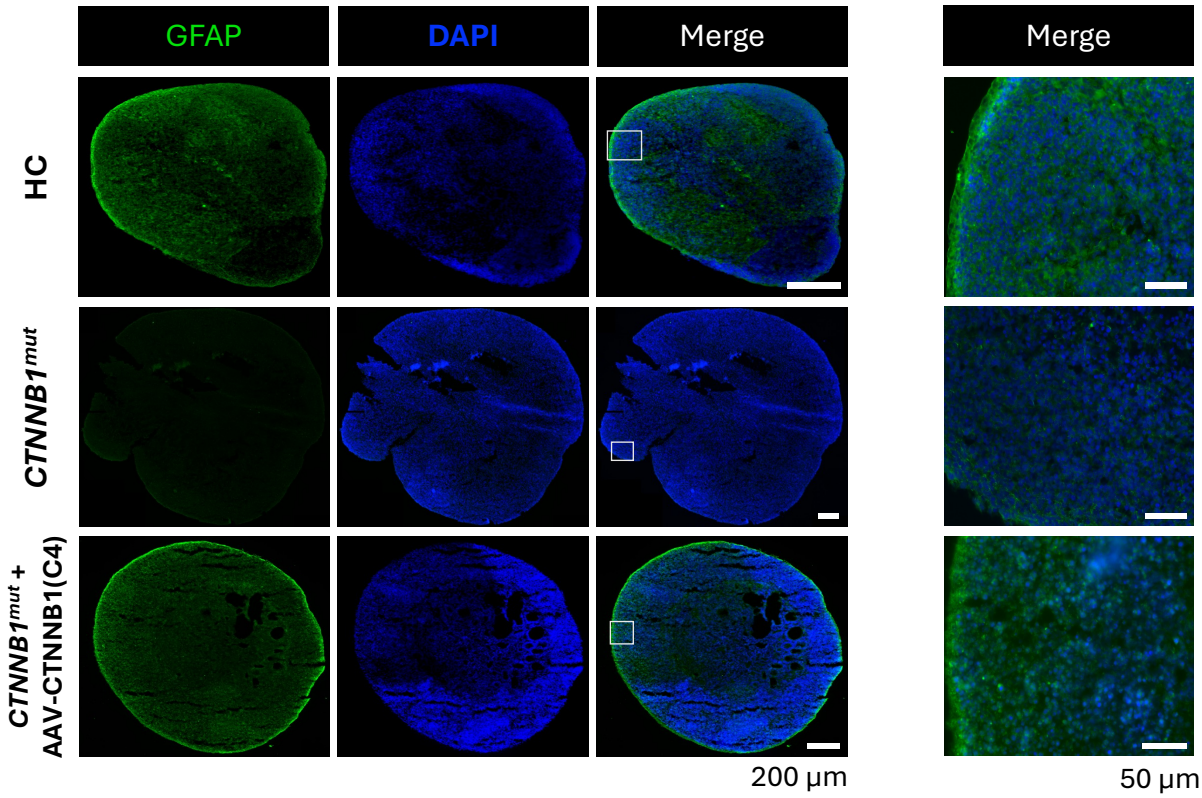
Supplementary Figure 4. Pathway-level assessment of oncogenic programmes and neuronal maturation-related transcriptional changes in cortical organoids. Bulk RNA-seq analysis was performed on cortical organoids derived from one CTNNB1 patient and two control lines (HC1 and HC2), under untreated (UN) and AAV-CTNNB1(C4)-treated conditions at T1 and T2. **(a)** Gene set variation analysis (GSVA) scores for pathways associated with proliferation and

oncogenic signaling, including E2F targets, G2M checkpoint, MYC targets, Wnt/β-catenin signaling, PI3K/AKT/mTOR, apoptosis and the p53 pathway. Points represent individual samples, with lines connecting matched conditions within each line and timepoint. **(b)** Heatmap of neuronal maturation-related genes. **(c)** Heatmap of glutamatergic synapse-related genes. Heatmaps show row-scaled expression values with hierarchical clustering. Annotation bars indicate timepoint, treatment condition, line and group. Across lines and timepoints, GSVA did not reveal a consistent treatment-associated increase in proliferation- or oncogenic pathway activity, while neuronal and synaptic gene expression patterns were preserved across conditions.

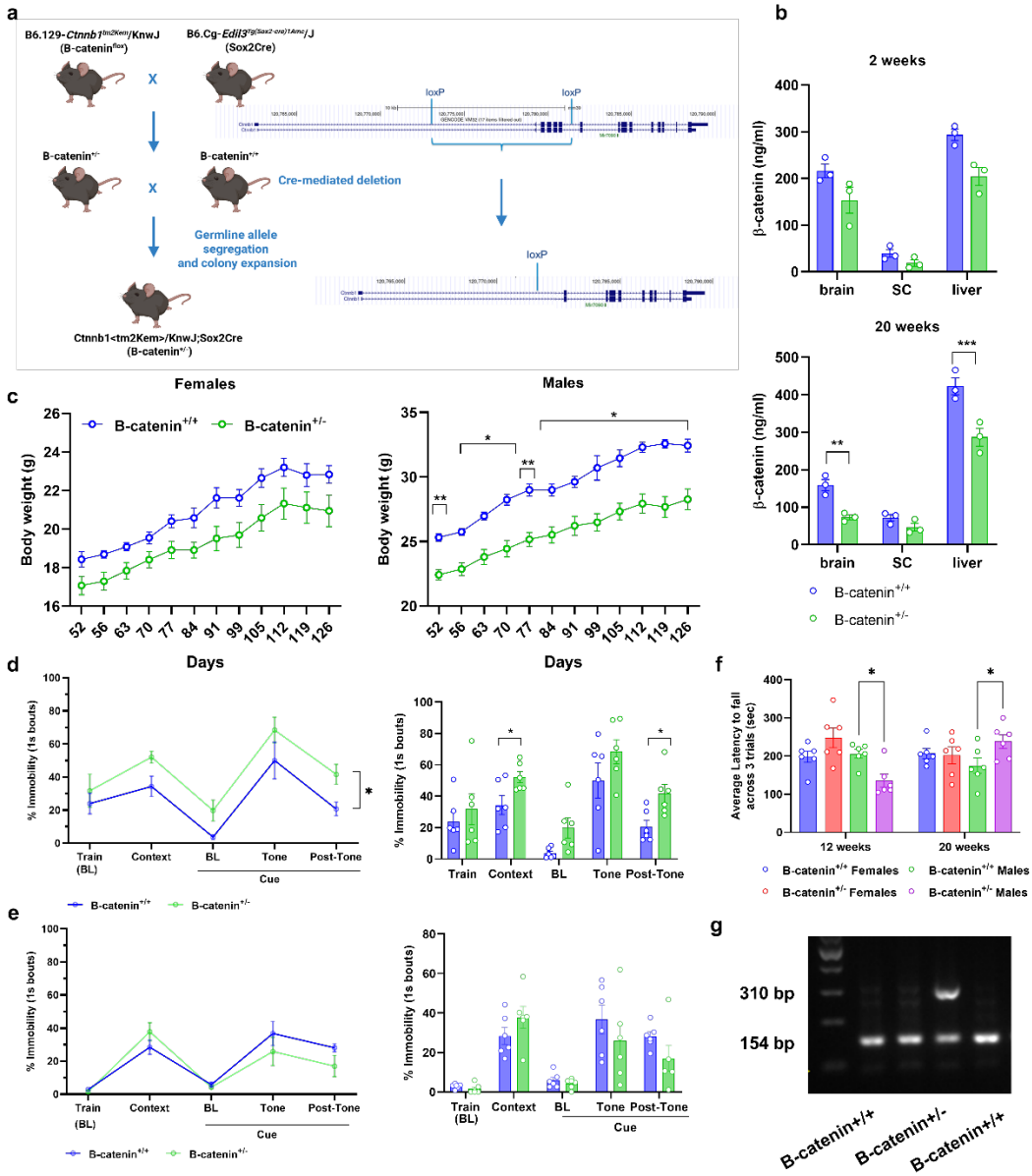


Supplementary Figure 5. Extended representative immunofluorescence images of healthy control (HC) and patient-derived (*CTNNB1^{mut}*) cortical brain organoids corresponding to Figure 1D. Patient-derived organoids were transduced with AAV2.7m8-CTNNB1(C4) at 1.00×10^{10} vector genomes (vg)/organoid and harvested two weeks post-transduction for analysis. Images show (**a**) β-catenin and (**b**) synaptophysin, with nuclei counterstained with

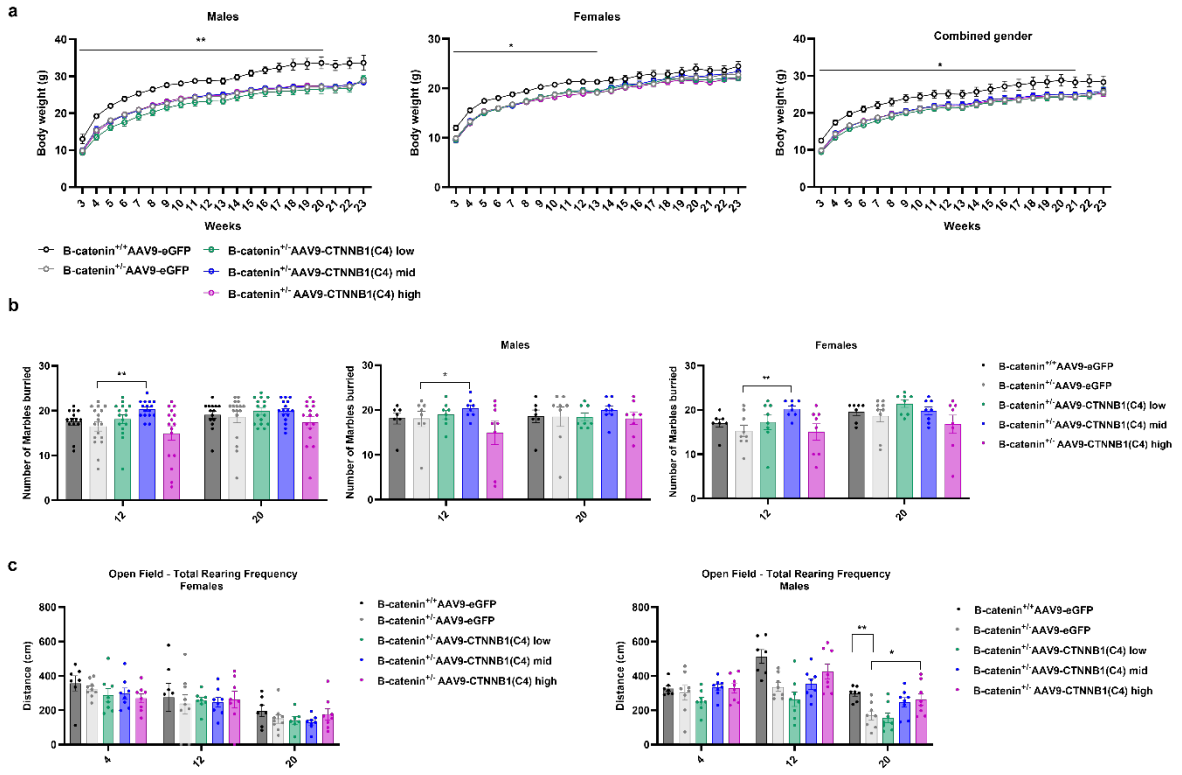
DAPI and merged channels. Higher-magnification views of each organoid are shown on the right. Scale bars 200 μm (overview) and 50 μm (zoom).



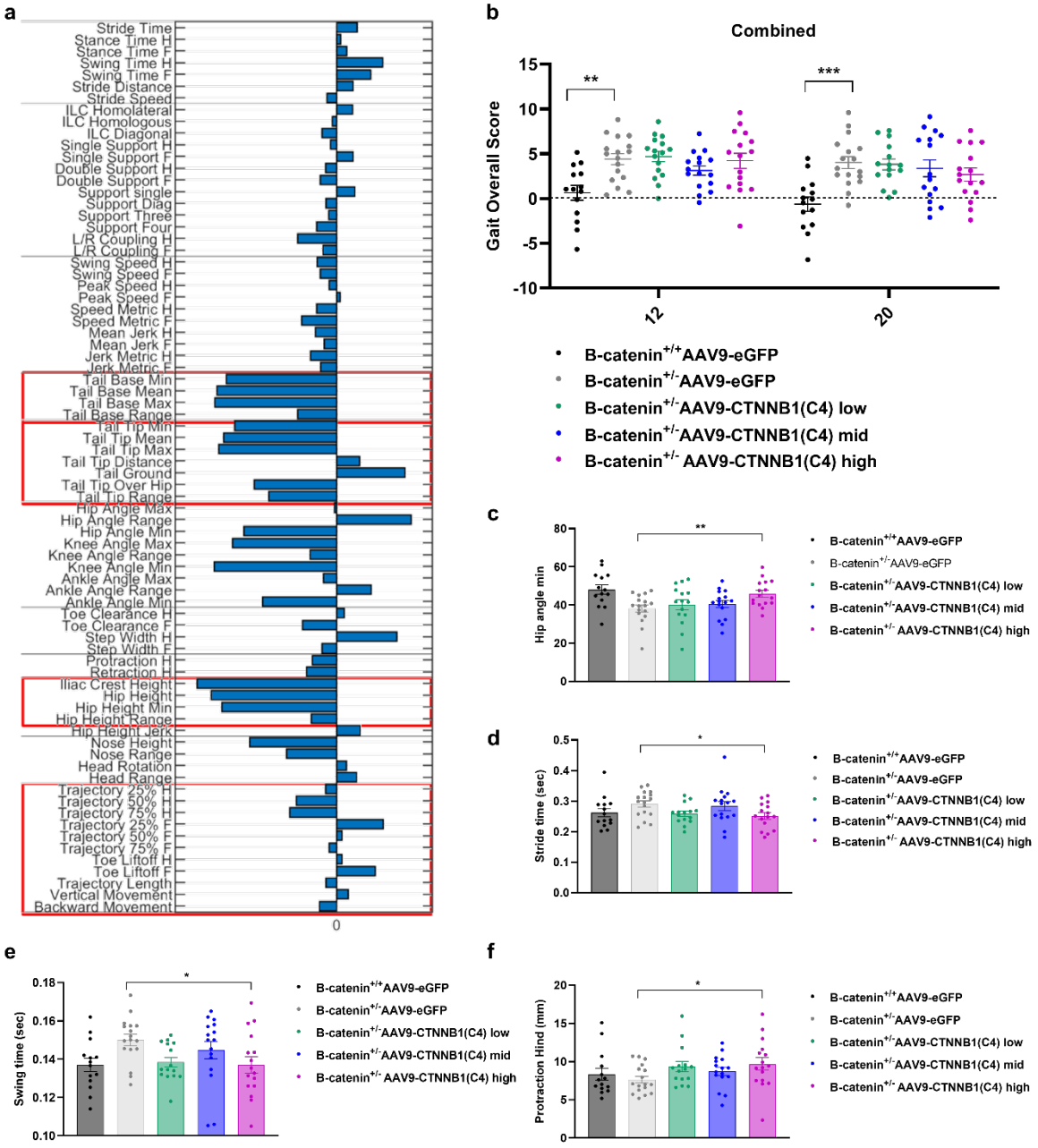
Supplementary Figure 6. Extended representative immunofluorescence images of healthy control (HC) and patient-derived (*CTNNB1*^{mut}) cortical brain organoids corresponding to Figure 1D. Patient-derived organoids were transduced with AAV2.7m8-CTNNB1(C4) at 1.00×10^{10} vector genomes (vg)/organoid and harvested two weeks post-transduction for analysis. Images show the Glial Fibrillary Acidic Protein (GFAP), with nuclei counterstained with DAPI and merged channels. Higher-magnification views of each organoid are shown on the right. Scale bars 200 µm (overview) and 50 µm (zoom).



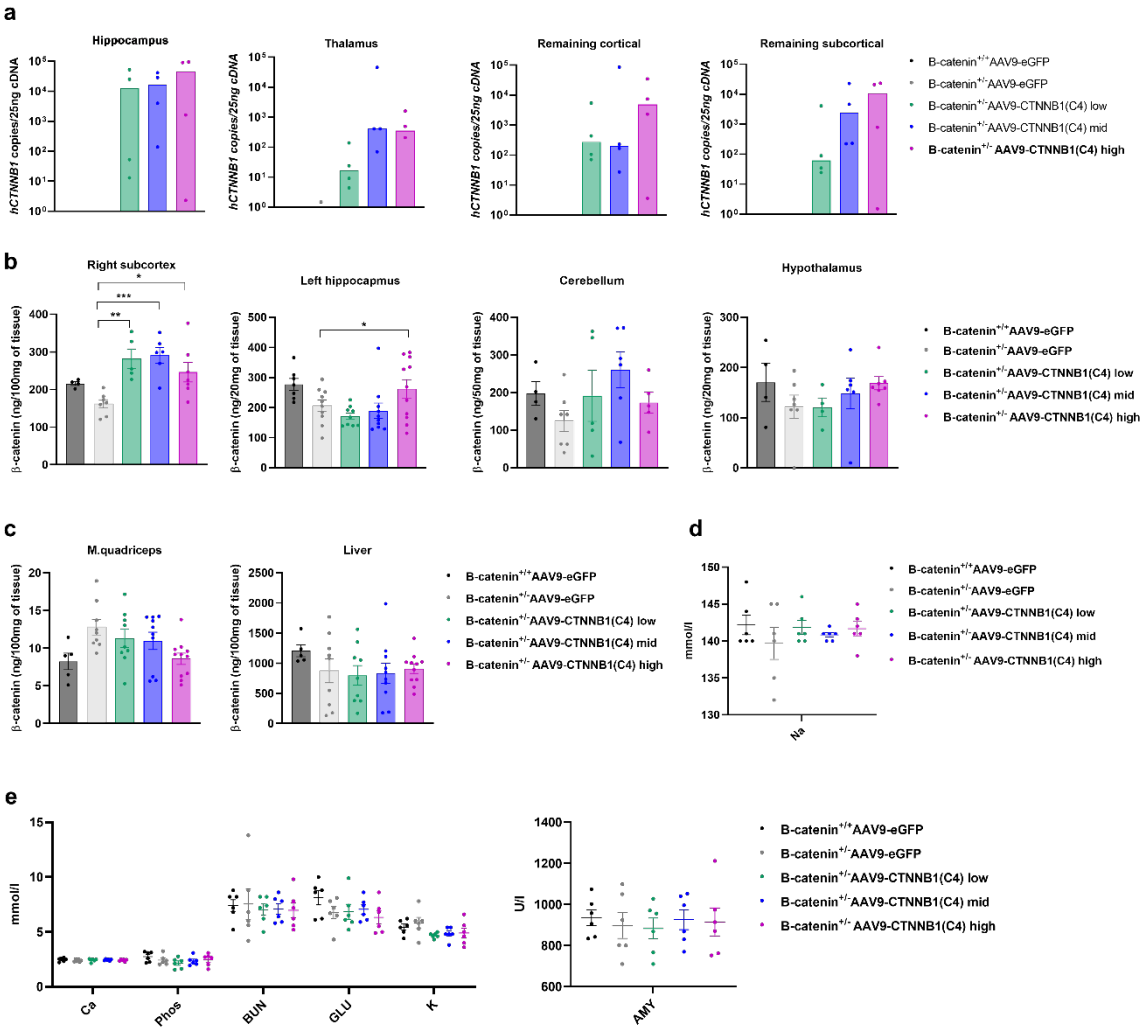
Supplementary Figure 7: CTNNB1 Syndrome preclinical mouse disease model generation and characterization. (a) Mouse CTNNB1 Syndrome preclinical disease model (β-catenin$^{+/}$) was developed by mating β-catenin^{flox} and Sox2Cre animals and subjected to characterization. (**b**) β-catenin protein determination via ELISA from mouse tissue harvested at different timepoints (mean ± SEM; n= 3/genotype). SC: Spinal Cord. (**c**) Body weight monitoring (mean ± SEM; n= 6/sex/genotype). (**d-e**) Fear conditioning determined at 20 weeks of age after 3 training sessions in female (**d**) and in male (**e**) mice (mean ± SEM; n= 6/sex/genotype). (**f**) Average latency to fall across three trials determined by rotarod test in at 12 and 20 weeks of age. (**g**) A representative 1.8% agarose gel electrophoresis depicting PCR amplicons from β-catenin$^{+/+}$ (154 bp) or β-catenin$^{+/-}$ mice (310 bp). Two-way ANOVA with Sidak's multiple comparisons test (* $p > 0.05$; ** $p > 0.01$; *** $p > 0.001$).



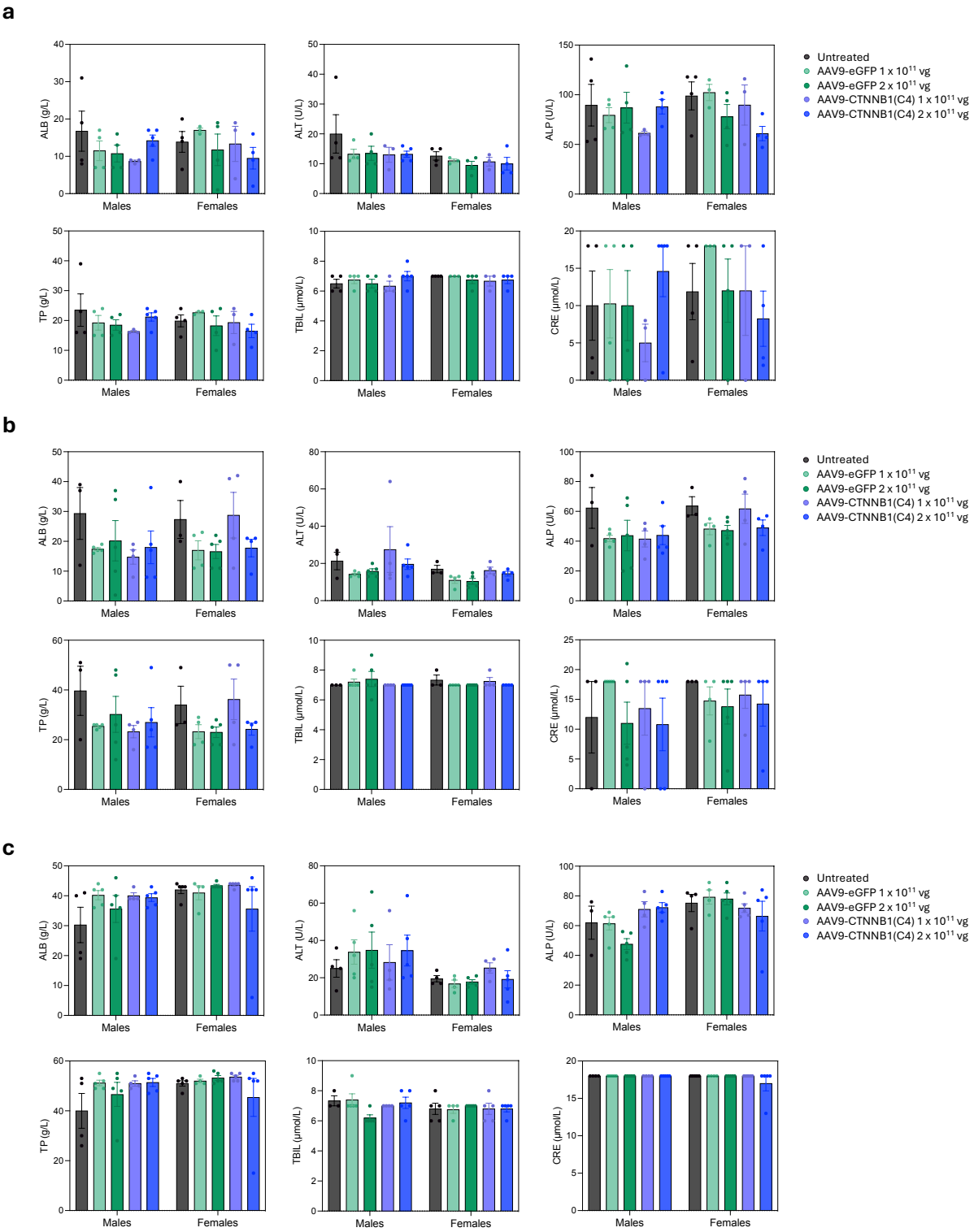
Supplementary Figure 8: Efficacy of AAV9-CTNNB1(C4) gene therapy in CTNNB1 Syndrome mouse model. Beta-catenin$^{+/-}$ (n= 8-9/sex/group) and β-catenin$^{+/+}$ (n= 7/sex) mice were stereotactically administered with AAV9-eGFP control vector (2.12×10^{10} vg/mouse) or AAV9-CTNNB1(C4) (low dose: 4.24×10^{9} vector genomes (vg)/mouse, mid dose: 2.12×10^{10} vg/mouse, high dose: 1.06×10^{11} vg/mouse. (**a**) Longitudinal body weight measurements of mice enrolled in the efficacy study, recorded from the time of vector administration through the study endpoint. (**b**) Number of marbles buried during marble burying test, evaluated in combined sexes and separately in males and females at 12 and 20 weeks of age (mean ± SEM; * $p < 0.05$; ** $p < 0.01$; Two-way ANOVA with Tukey's multiple comparisons test). (**c**) Total Rearing Frequency determined in Open Field Test at depicted time points of the study (mean ± SEM; * $p < 0.05$; ** $p < 0.01$; Two-way ANOVA with Tukey's multiple comparisons test).



Supplementary Figure 9: Kinematic Gait Analysis parameters in mouse disease model efficacy study. (a) The discriminant vector, consisting of weights for 76 individual kinematic parameters. The discriminant vector bar graph illustrates the overall kinematic fingerprint of the model, generated by comparing β-catenin$^{+/-}$ AAV9-eGFP and β-catenin$^{+/+}$ AAV9-eGFP controls. Gait parameters most prominently altered in the model are highlighted with a red rectangle. **(b)** Overall Kinematic Gait score for combined sexes, assessed at 12 and 20 weeks of age. (**c-f**) Specific Gait Parameters, affected by treatment (n= 8-9/sex/group; mean ± SEM; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.01$ unpaired *t*-test).

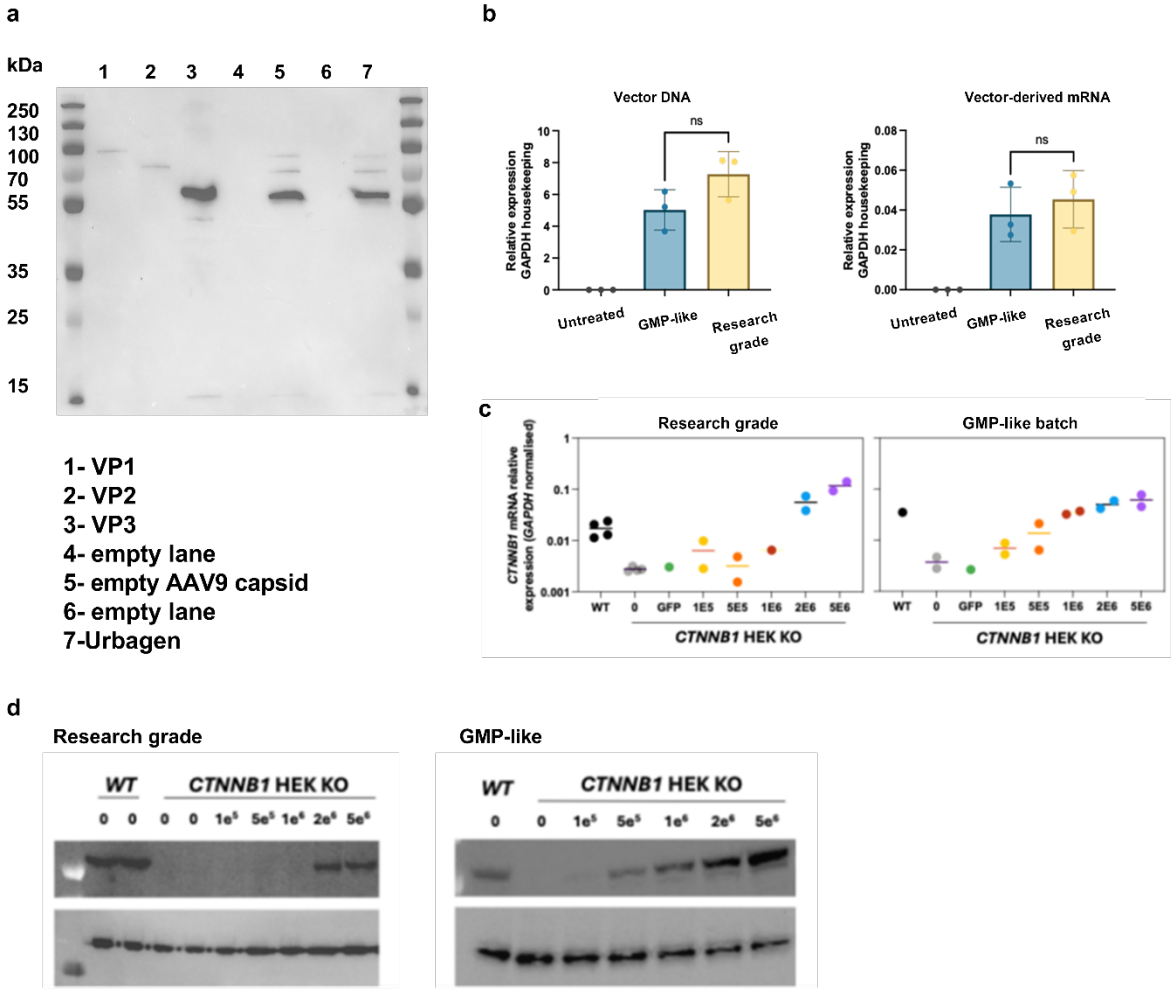


Supplementary Figure 10: Therapeutic effect of AAV9-CTNNB1(C4) in the CTNNB1 Syndrome mouse model. (a) Determination of human *CTNNB1* mRNA levels across different regions of the brain in mice via RT-qPCR, normalized to mouse *Gapdh* (n= 4/group; median). **(b-c)** Human β-catenin protein levels determined via ELISA across distinct regions of the mouse brain (**b**) and in peripheral tissues (**c**) at the end of the study via RT-qPCR (mean ± SEM; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, Two-way ANOVA with Tukey's multiple comparisons test; n= 4-7/group). (**d-e**) Biochemical blood profile assessed at study endpoint; sodium (Na) (**d**), calcium (Ca), phosphate (Phos), blood urea nitrogen (BUN), glucose (GLU), potassium (K) and amylase (AMY) (**e**) (n= 6-7/group).

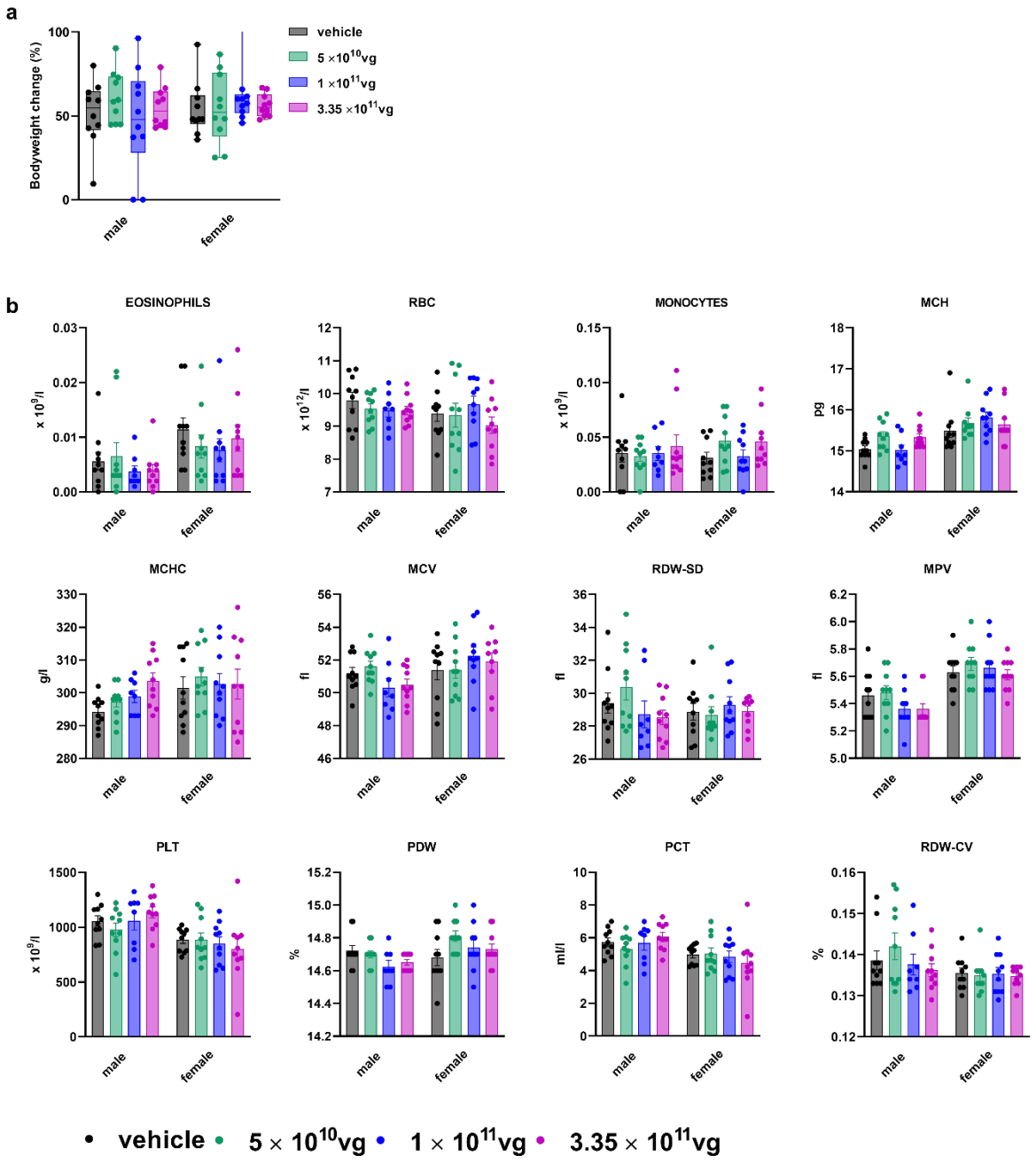


Supplementary Figure 11. Plasma biochemical profile in C57BL/6J mice from the 8-month toxicology study. Wild-type mice were administered either 1.06×10^{11} or 2.25×10^{11} vector genomes (vg) of the selected AAV9-CTNNB1(C4) or the AAV9-eGFP control vector and monitored for eight months. Untreated wild-type mice (n= 5/group) served as controls. Analysis of biochemical parameters (**a-c**) were performed (**a**) one week, (**b**) three months, and (**c**) eight months post-administration. Data are shown as mean ± SEM. ANOVA > 0.05. ALB:

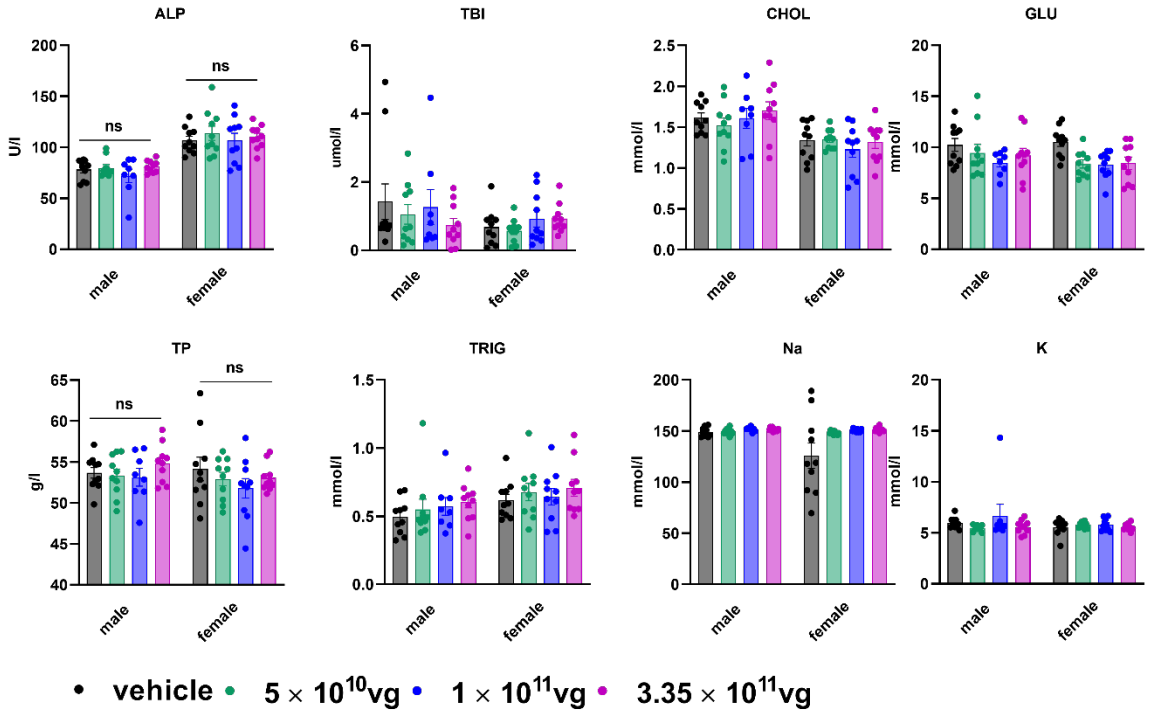
Albumin; ALT: Alanine aminotransferase; ALP: Alkaline Phosphatase; TP: Total protein, TBIL: Total bilirubin; CRE: Creatinine.



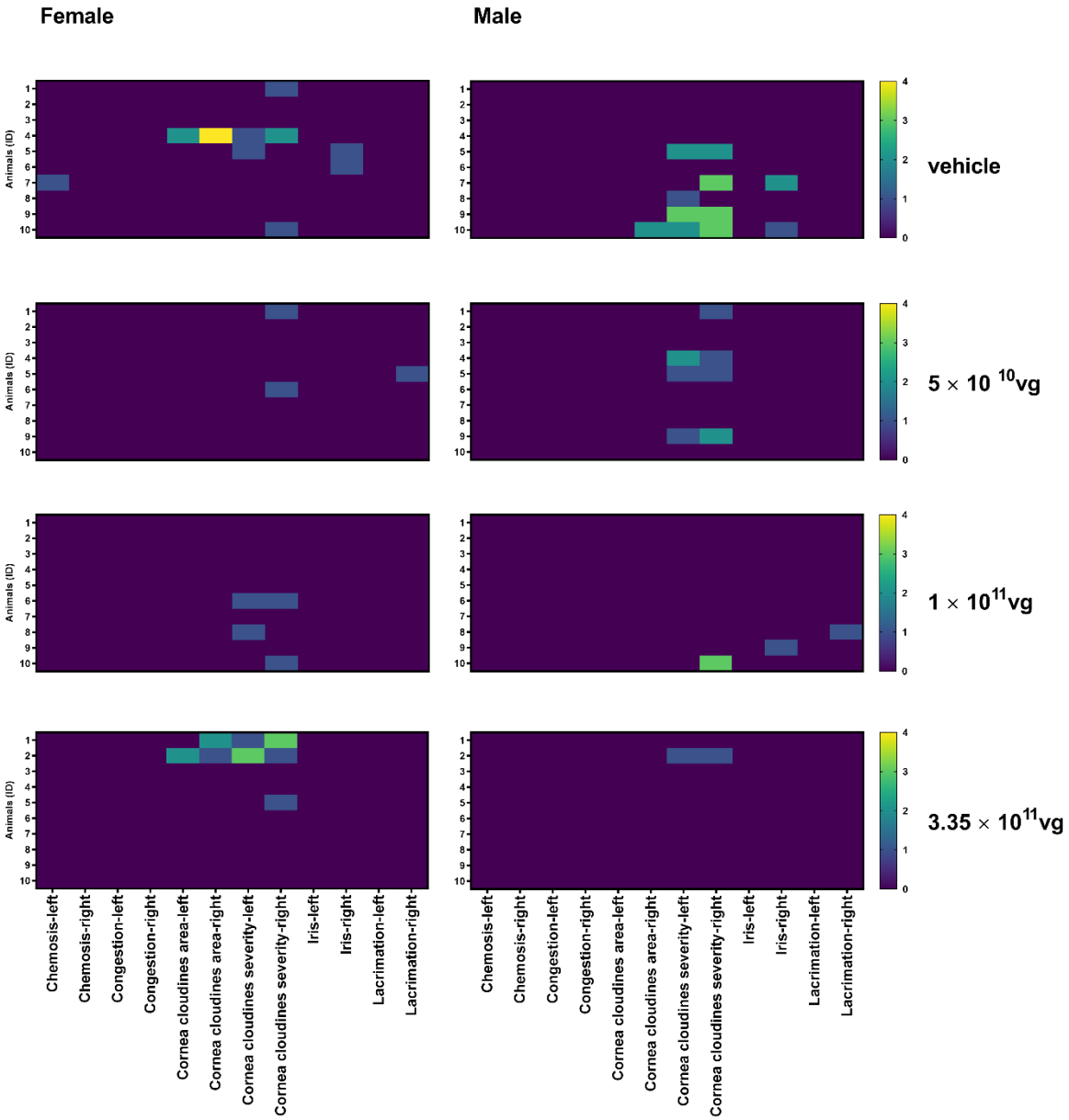
Supplementary Figure 12: Urbagen identity confirmation. (a) Identity confirmation of Urbagen tested via SDS-PAGE and western blot analysis to confirm the identity of the AAV9 viral proteins (VP1, VP2, VP3). **(b)** Research grade and GMP-like batch comparison in vector DNA and in vector-derived mRNA. HEK293T cells were transduced with and MOT of 2.00 × 10^6 vg/cell and collected 72 hours post-transduction for subsequent analysis by RT-qPCR (n= 3; unpaired *t*-test). **(c-d)** Potency assay of the research grade and GMP-like Urbagen, determined by RT-qPCR (**c**) and western blot (**d**). CTNNB1 KO HEK293 cells were transduced with increasing concentrations and analyzed after 96 h.



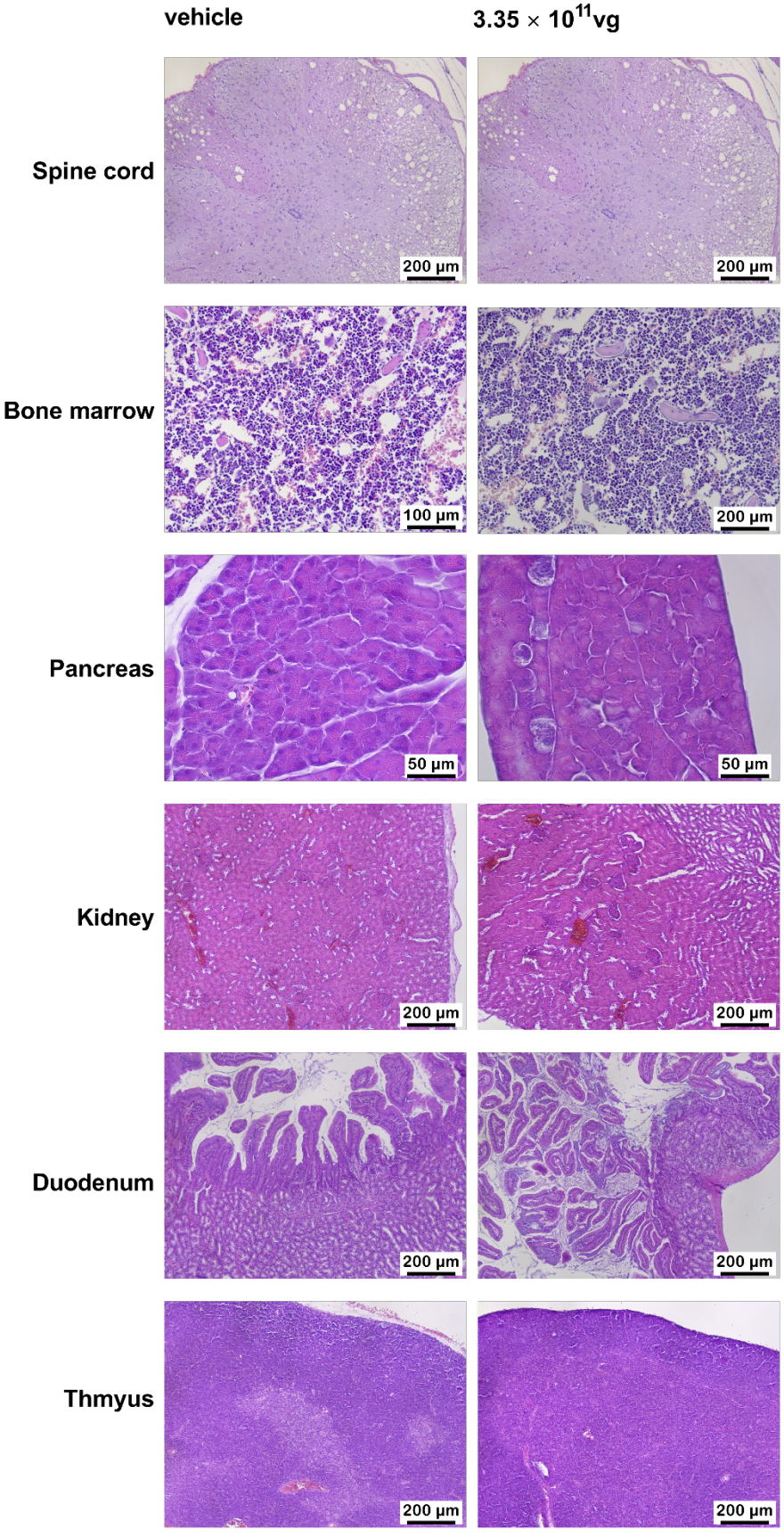
Supplementary Figure 13: Clinical assessment outcomes from the 90-day toxicology study. Urbagen was ICV administered to wild-type mice at doses of 5.00 × 10^{10}, 1.00 × 10^{11}, and 3.35 × 10^{11} vg/mouse (n= 10/sex/dose). (**a**) Body weight change. (**b**) Hematology analysis at the end of the study. Data represented as mean ± SEM RBC: Red Blood Cell count; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; MCV: Mean Corpuscular Volume; RDW-SD: Red Cell Distribution Width – Standard Deviation; RDW-CV: Red Cell Distribution Width – Coefficient of Variation; PLT: Platelet count; MPV: Mean Platelet Volume; PDW: Platelet Distribution Width; PCT: Plateletcrit.



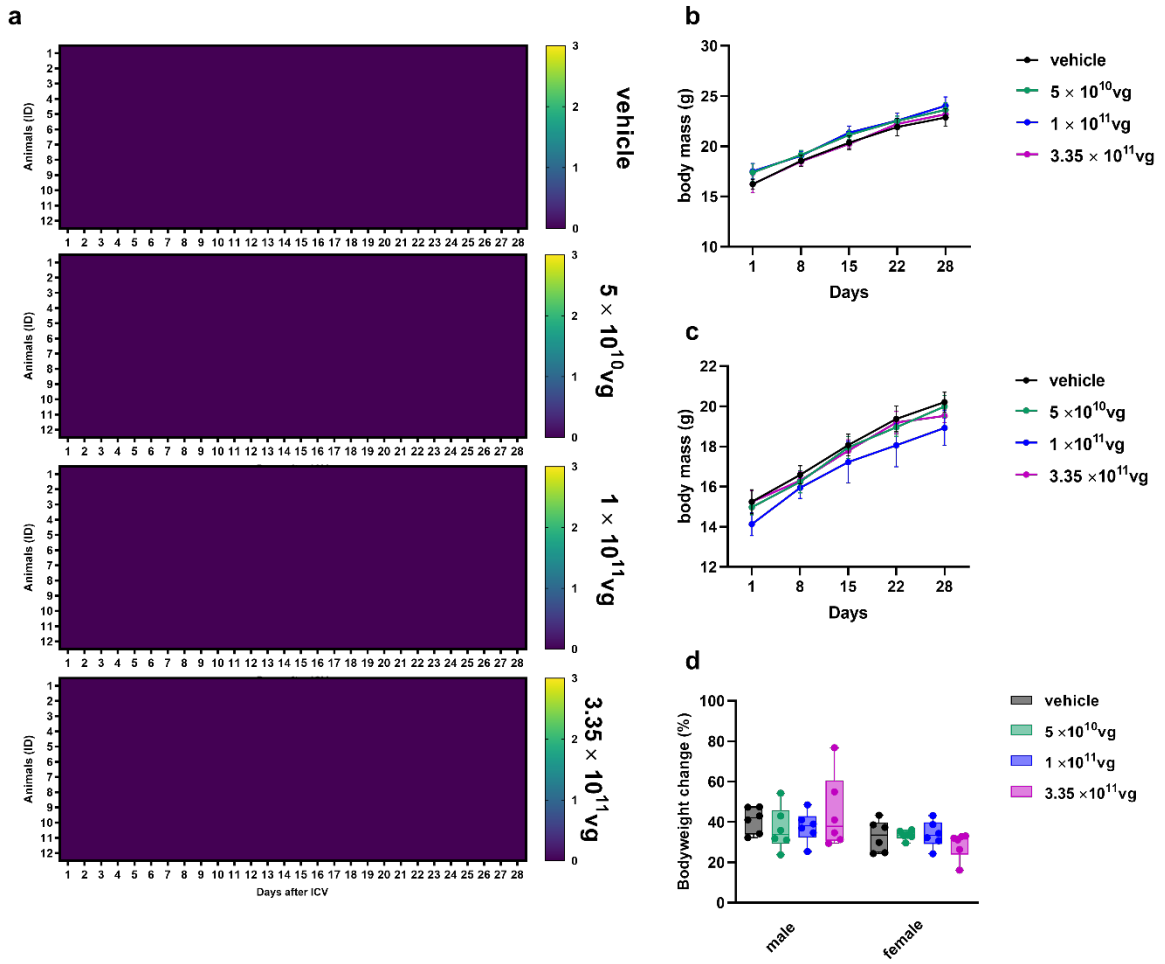
Supplementary Figure 14: Blood biochemical profile from the 90-day toxicology. Wild-type mice were administered Urbagen at 5.00×10^{10}, 1.00×10^{11}, and 3.35×10^{11} vg/mouse (n= 10/sex/dose) through the ICV route. At the end of the study biochemical analysis of the blood was performed. Data represented as mean ± SEM. ALP: Alkaline Phosphatase; TBI: Total Bilirubin; CHOL: Cholesterol; GLU: Glucose; TP: Total Protein; TRIG: Triglycerides; Na: Sodium; K: Potassium.



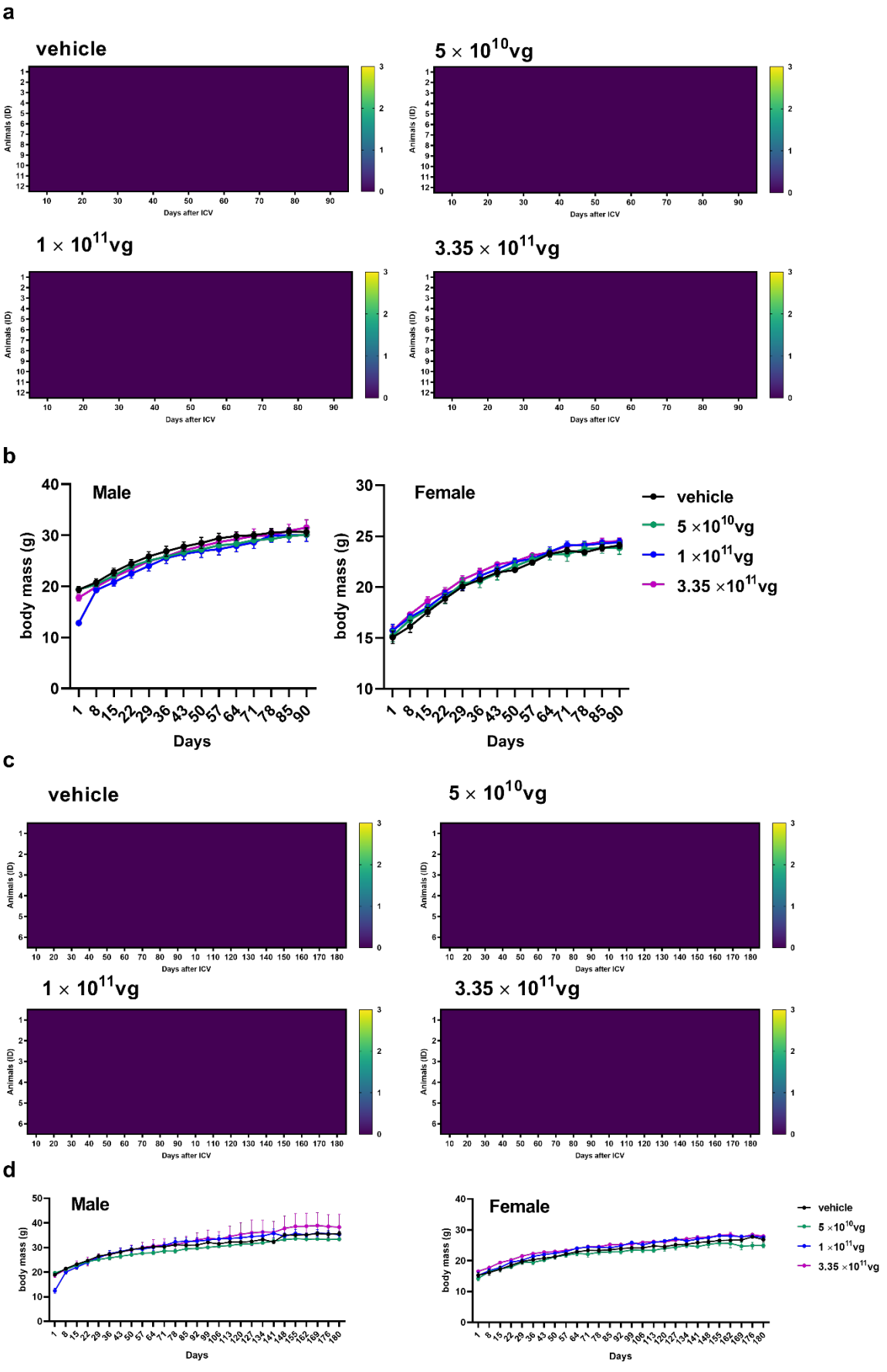
Supplementary Figure 15: Ophthalmological examination at the end of the 90-day toxicology study in mice. Mice (n= 10/sex, y-axis) were ICV inoculated with either 5.00×10^{10}, 1.00×10^{11}, or 3.35×10^{11} vg/mouse of Urbagen and eye examination was performed 90 days post-administration.


vehicle
3.35 × 10^11vg
Spine cord
Bone marrow
Pancreas
Kidney
Duodenum
Thmyus
200 μm
100 μm
50 μm

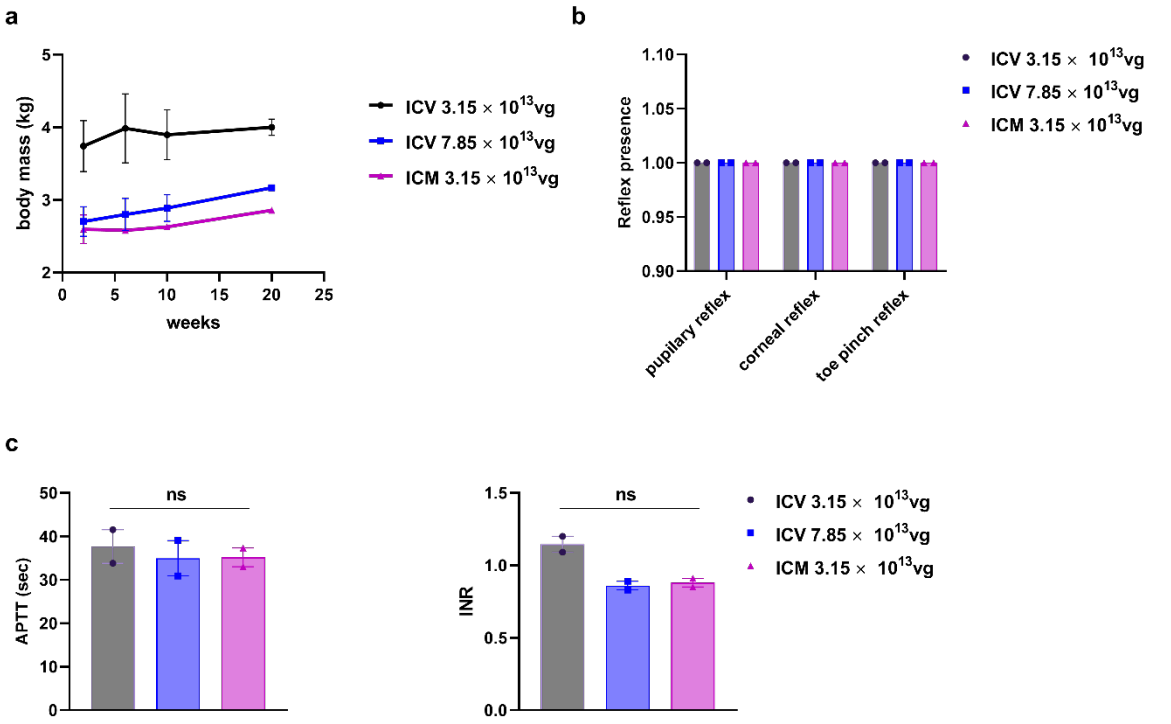
Supplementary Figure 16: Representative histopathology images from the 90-day toxicology study in mice. Hematoxylin and eosin (H&E)-stained tissue sections, harvested at the end of the study. Scale bars: ~50-200µm.



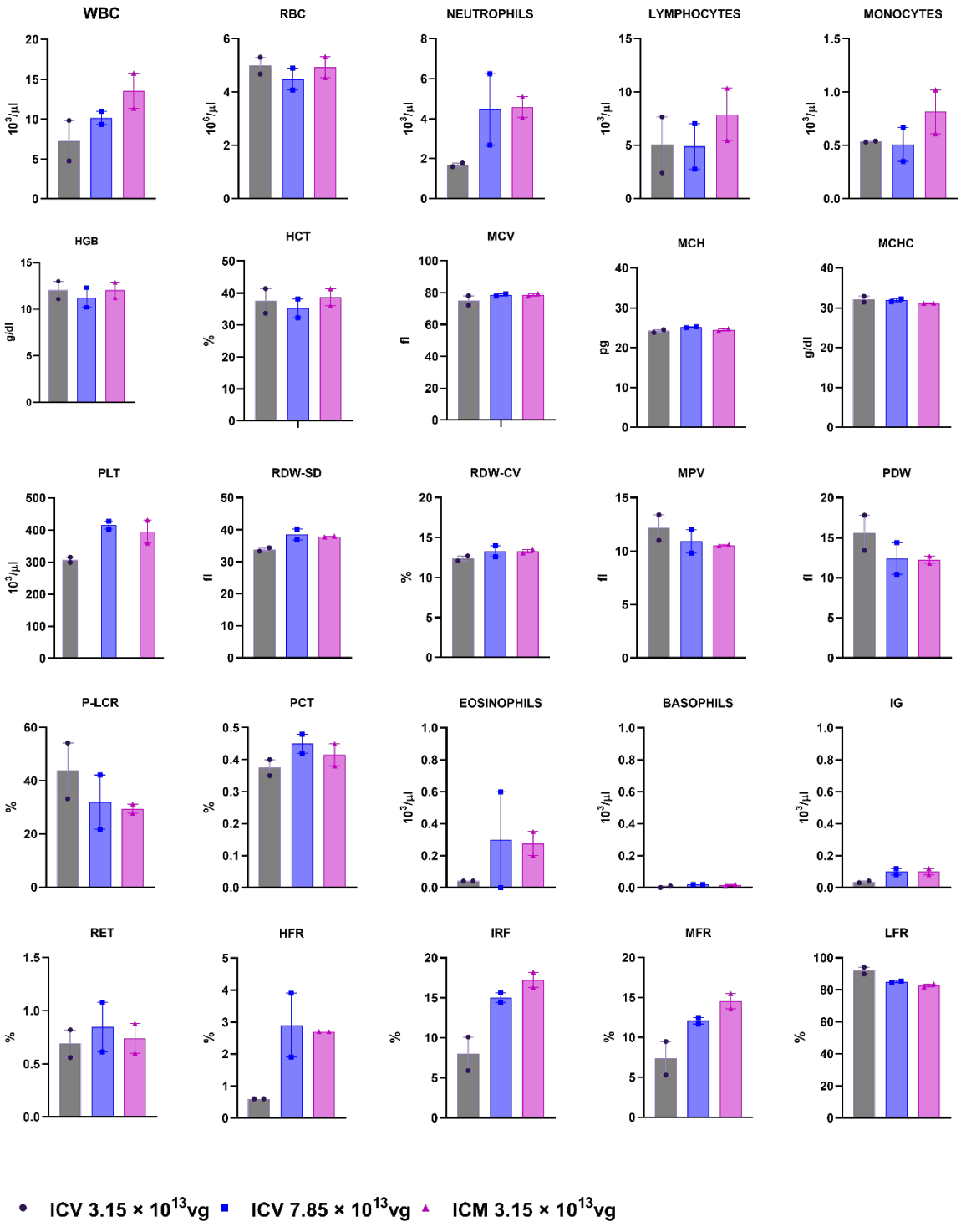
Supplementary Figure 17: Clinical observation of mice in 28-day biodistribution study (BDS). Urbagen was administered at low (5.00×10^{10} vg/mouse), mid (1.00×10^{11} vg/mouse), and high (3.35×10^{11} vg/mouse) doses through the ICV route into wild-type mice (n= 6/sex/dose). (**a**) Daily clinical observation scores of mice from the 28-day BDS. (**b-c**) Weight curve of male (**b**) and female (**c**) mice throughout the study. (**d**) Percentage of body weight change for all animals throughout the 28-day BDS. Data represented as mean ± SEM.


a
vehicle
5 × 10^10vg
1 × 10^11vg
3.35 × 10^11vg
Animals (ID)
Days after ICV
b
Male
Female
body mass (g)
Days
vehicle
5 ×10^10vg
1 ×10^11vg
3.35 ×10^11vg
c
vehicle
5 × 10^10vg
1 × 10^11vg
3.35 × 10^11vg
Animals (ID)
Days after ICV
d
Male
Female
body mass (g)
Days
vehicle
5 ×10^10vg
1 ×10^11vg
3.35 ×10^11vg

Supplementary Figure 18: Clinical observation of mice in 90- and 180-day biodistribution studies (BDS). Urbagen was administered through the ICV route to wild-type mice at three dose levels (n= 6/sex/dose for 90-day and n= 3/sex/dose for 180-day). Daily clinical observation score (**a**) and weight curve **(c)** of mice from the 90-day BDS. Daily clinical observation score and weight curve (**d**) of mice from the 180-day BDS. Data represented as mean ± SEM.

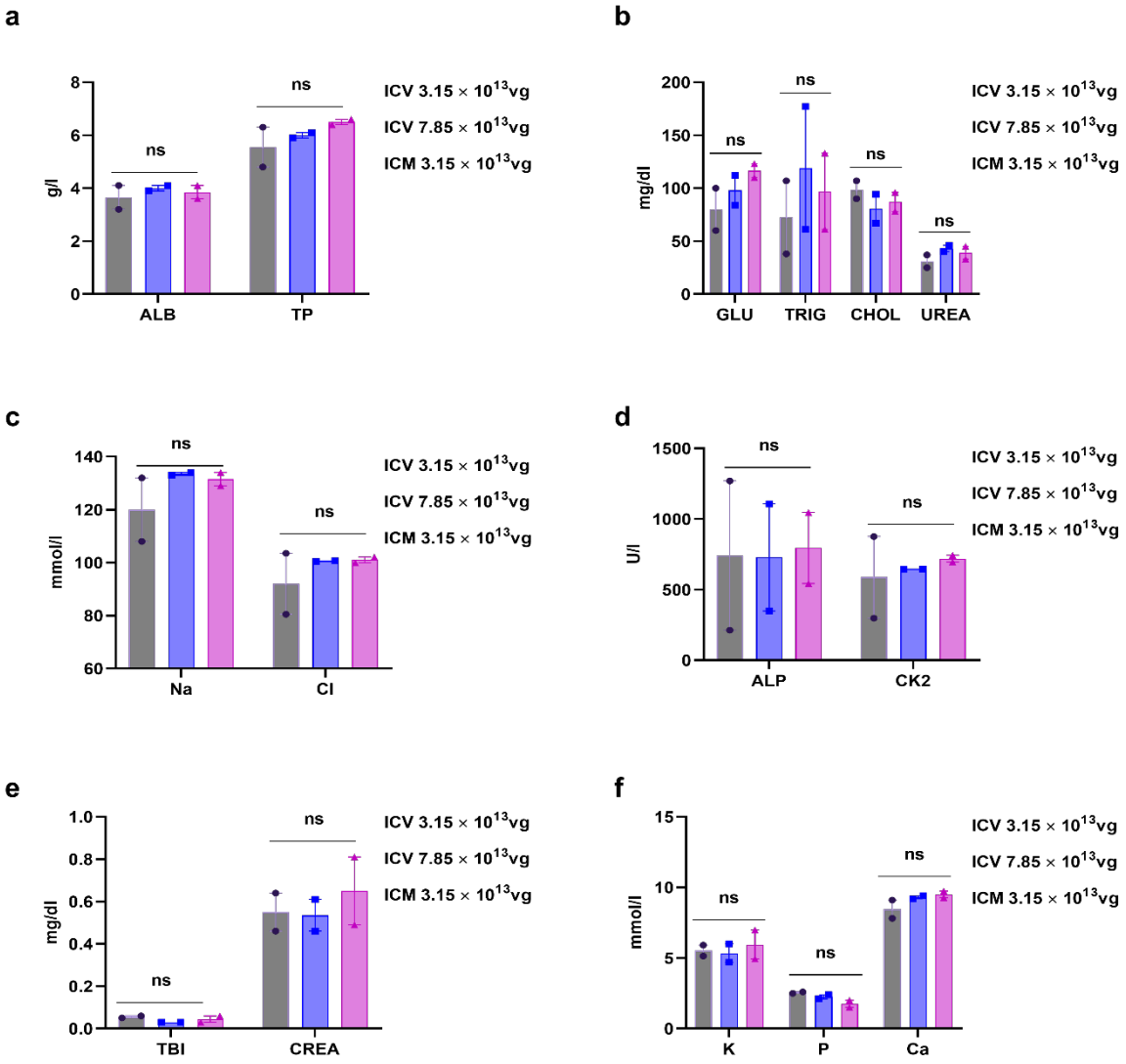


Supplementary Figure 19: Clinical observations of *Macaca fascicularis* in the biodistribution and toxicology study. Nonhuman primates (NHPs) were treated with either 3.15×10^{13} or 7.85×10^{13} vg of Urbagen via stereotactic brain surgery using two different delivery approaches: biventricular ICV, or intracisterna magna (ICM). (**a**) Body weight monitoring throughout the study post treatment. (**b**) Weekly assessment of reflexes, with presence scored as 1 and absence scored as 0. (**c-d**) Coagulation factors measured 90 days post-administration (n= 2/group; mean ± SEM, one-way ANOVA). APTT: Activated Partial Thromboplastin Time; INR: International Normalized Ratio.

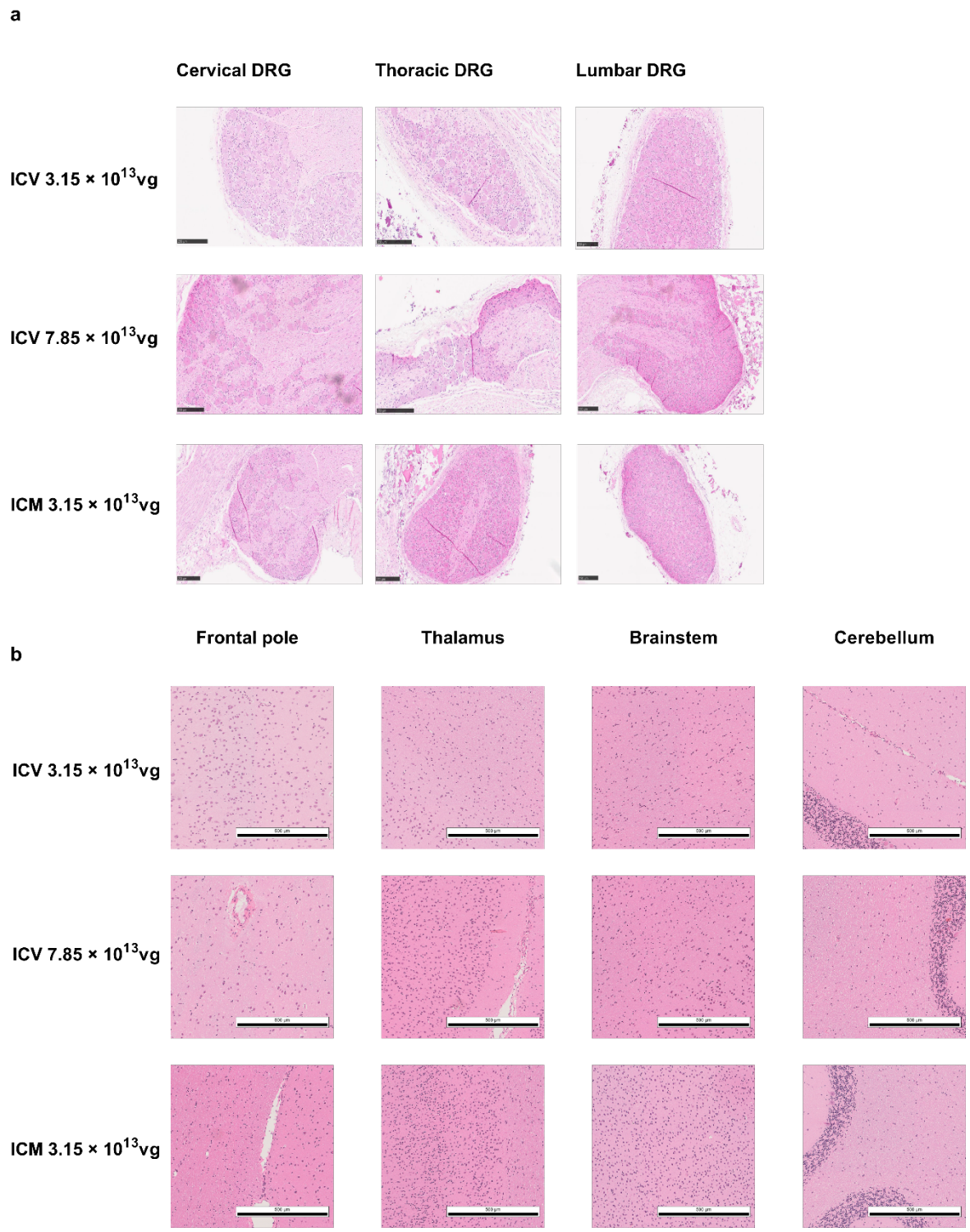


Supplementary Figure 20: Hematology analysis of *Macaca fascicularis* in the biodistribution and toxicology study. Nonhuman primates (NHPs) were treated with either 3.15×10^{13} or 7.85×10^{13} vg of Urbagen via stereotactic brain surgery using two different delivery approaches: biventricular ICV, or intracisterna magna (ICM). Clinical chemistry was performed 90 days post-administration (n= 2/group; mean ± SEM). Data were analyzed using two-way ANOVA followed by Tukey's multiple comparison test. WBC: White Blood Cells;

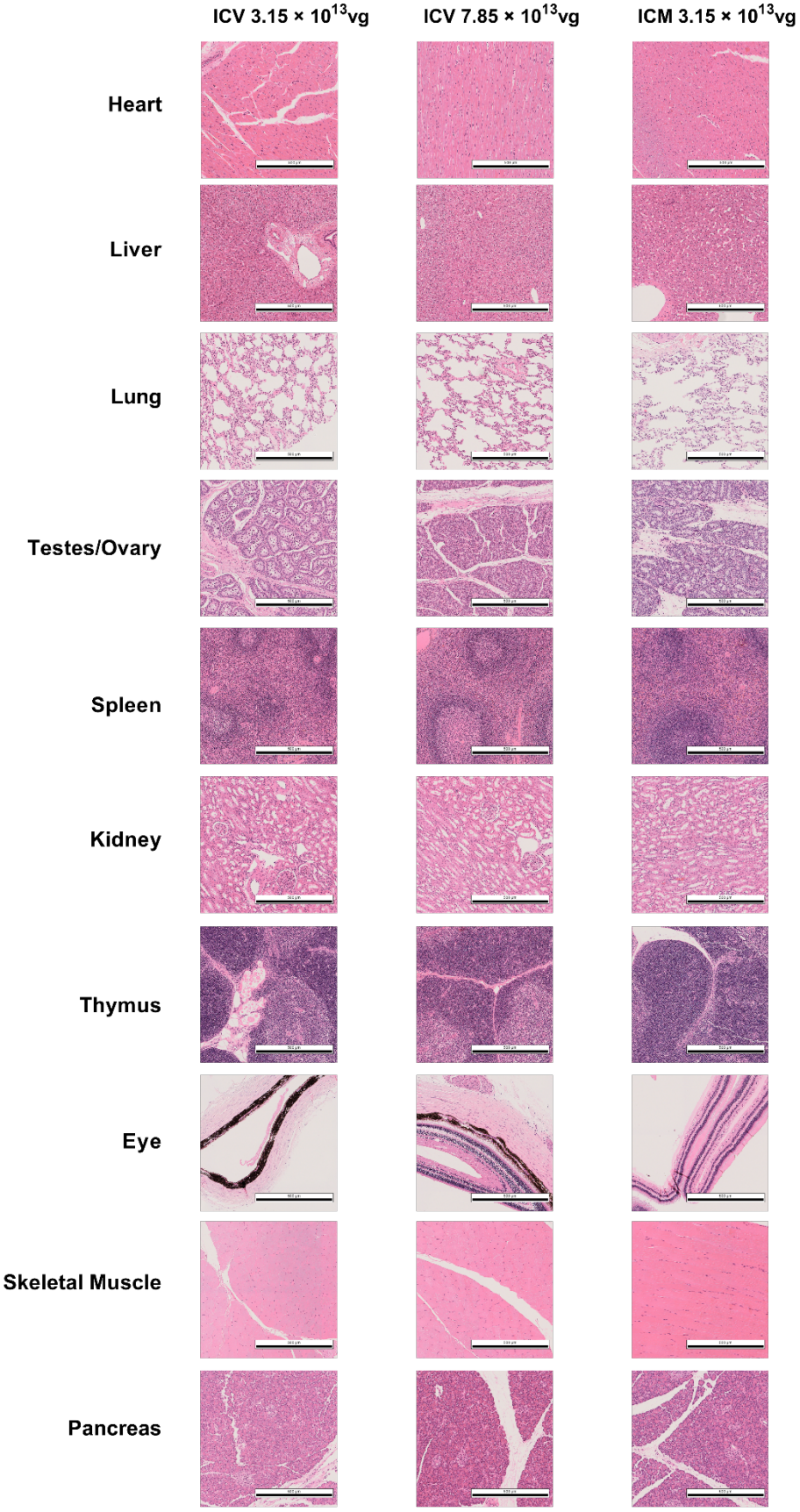
RBC: Red Blood Cells; HGB: Hemoglobin; HCT: Hematocrit, MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; PLT: Platelets; RDW-SD: Red Cell Distribution Width – Standard Deviation; RDW-CV: Red Cell Distribution Width – Coefficient of Variation; MPV: Mean Platelet Volume; PDW: Platelet Distribution Width; P-LCR: Platelet Large Cell Ratio; PCT: Procalcitonin; IG: Immature Granulocytes; RET: Reticulocytes; HFR: Highly Fluorescent Reticulocyte; IRF: Immature Reticulocyte Fraction; MFR: Medium Fluorescence Ratio; LFR: Low Fluorescence Ratio.



Supplementary Figure 21: Biochemical analysis of *Macaca fascicularis* in the biodistribution and toxicology study. Non-human primates (NHPs) were treated with either 3.15×10^{13} or 7.85×10^{13} vg of Urbagen via stereotactic brain surgery using two different delivery approaches: biventricular ICV, or intracisterna magna (ICM). Biochemical analysis was performed 90 days post-administration (n= 2/group; mean ± SEM). Two-way ANOVA with Tukey's multiple comparisons test. ALB: Albumin; TP: Total Protein; GLU: Glucose, TRIG: Triglyceride; CHOL: Cholesterol; Na: Sodium; ALP: Alkaline Phosphatase; CK2: Creatinine kinase 2; TBI: Total bilirubin; CREA: Creatinine; K: Potassium; P: Phosphorus, Ca: Calcium.



Supplementary Figure 22: Histology of the Central Nervous System (CNS) in *Macaca fascicularis* following Urbagen administration. Representative H&E sections of **(a)** dorsal root ganglion (DRG) and (**b**) brain. Scale bars: ~250 µm (DRG) and ~500 µm (brain).


ICV 3.15 × 10^13vg
ICV 7.85 × 10^13vg
ICM 3.15 × 10^13vg
Heart
Liver
Lung
Testes/Ovary
Spleen
Kidney
Thymus
Eye
Skeletal Muscle
Pancreas

Supplementary Figure 23: Histology of the peripheral tissues in *Macaca fascicularis* following Urbagen administration. Representative H&E stained sections are shown. Scale bar: ~500µm.