



Neonatal ICV Delivery and Longitudinal NF-L Monitoring as Tools for Early CNS Intervention in Preclinical Studies

Irene Schilcher, Martin Haering, Balasz Dobrovich, Tina Loeffler, Stefan Ebner-Benke, Magdalena Daurer, Livia Breznik, Manuela Prokesch
Scantox Neuro GmbH, Grambach, Austria



For further information and inquiries contact office-austria@scantox.com

Introduction

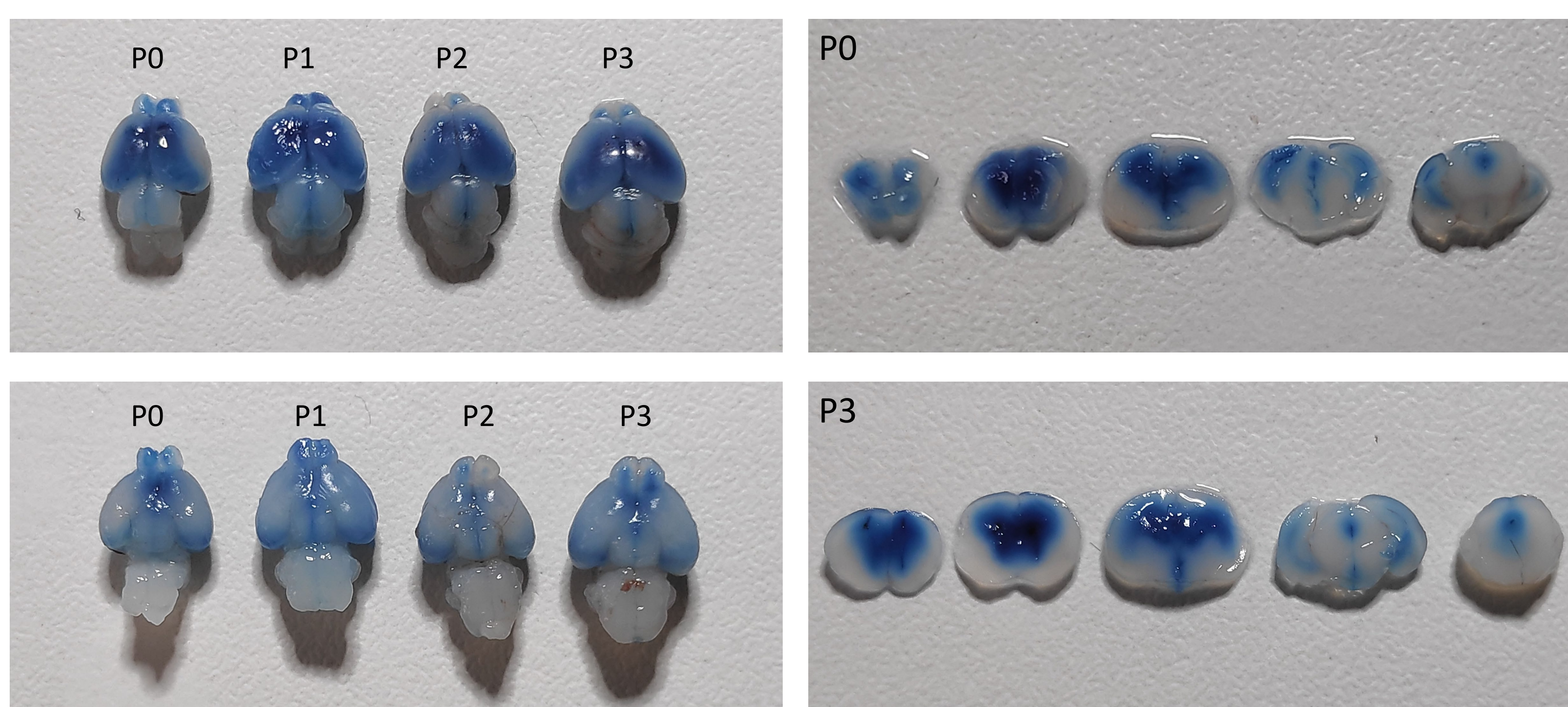
Lysosomal storage diseases (LSDs) such as Gaucher disease, Niemann-Pick type C1, Pompe disease, and Krabbe disease often present with early neurological involvement and rapid progression. Mouse models including 4L/PS-NA and NPC1^{-/-} recapitulate key clinical features, notably early neurodegeneration, reduced survival, and elevated neurofilament light chain (NF-L) levels in plasma and cerebrospinal fluid (CSF). As a sensitive biomarker of axonal injury, NF-L enables real-time monitoring of disease progression and therapeutic response. Because early CNS pathology is central to LSDs, presymptomatic intervention is critical. To support this need, neonatal intracerebroventricular (ICV) delivery provides broad CNS access for diverse therapeutic modalities. This poster highlights how integrating neonatal ICV administration with longitudinal NF-L sampling enhances the translational value of preclinical LSD studies. This poster represents technical feasibility of both, neonatal ICV injection and longitudinal CSF collection.

Methods

To assess the precision and CNS-wide distribution achieved by neonatal intracerebroventricular delivery—a method essential for early intervention—early postnatal pups (P0–P3) received ICV injections of either Evans blue dye or AAV-GFP. Directly after injection brains were macroscopically investigated for Evans Blue distribution, after long term treatment AAV9 transduction and distribution was visualized via immunohistochemical methods.

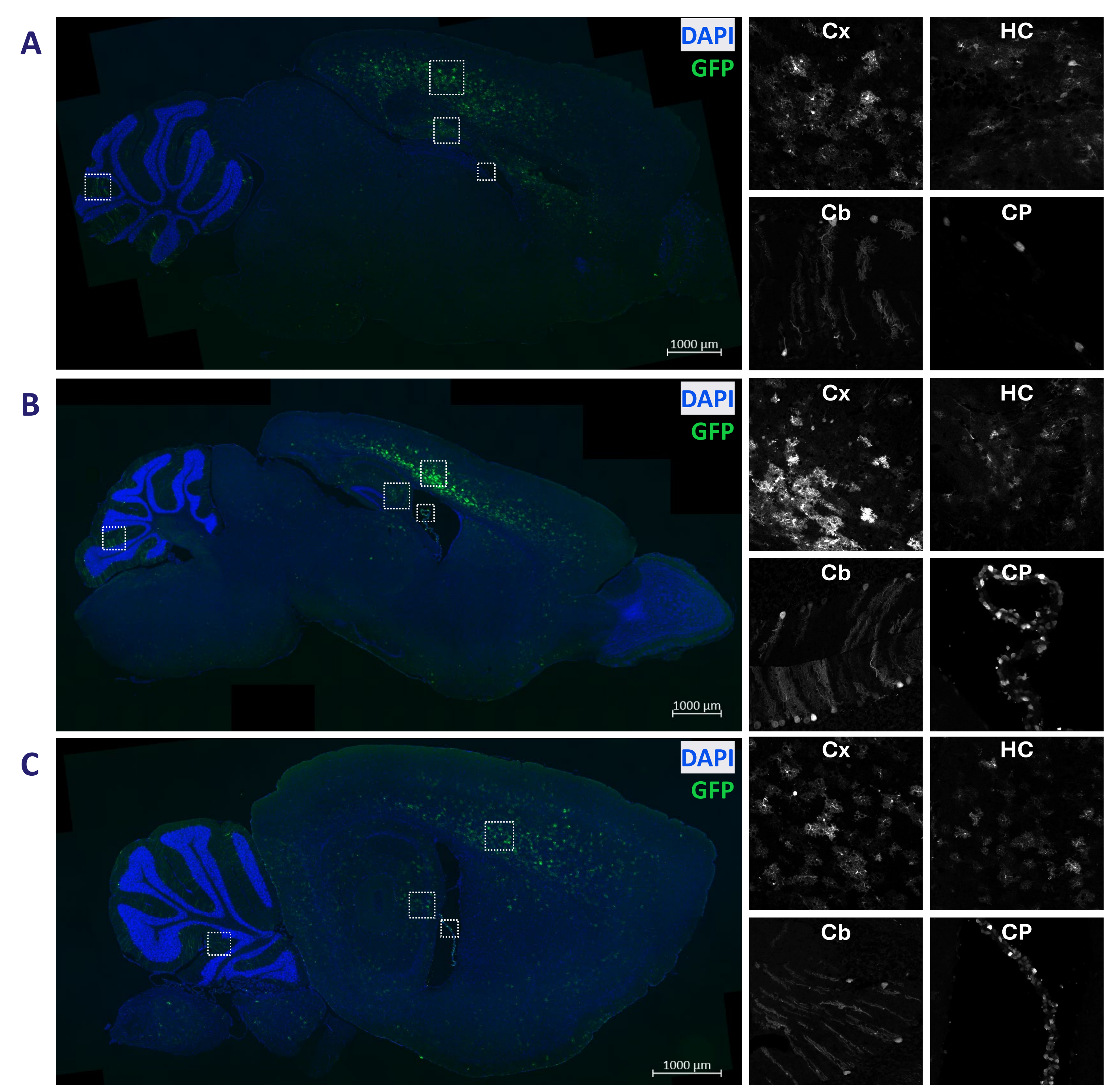
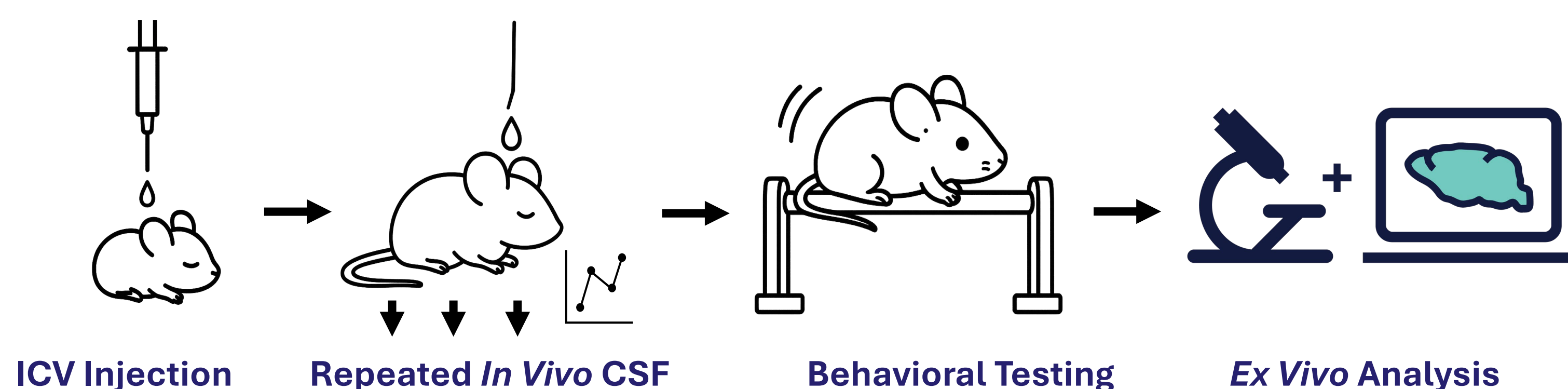
To demonstrate early neuronal damage in LSDs context, CSF was collected longitudinally from NPC1^{-/-} and 4L/PS-NA mice, along with their wild type littermates (n = 8 per group), using a specialized *in vivo* sampling technique at three defined time points. NF-L concentrations were quantified using the NF-Light® ELISA (UmanDiagnostics), a validated assay for both human clinical and murine samples.

Precision and Distribution Achieved by Neonatal ICV Delivery

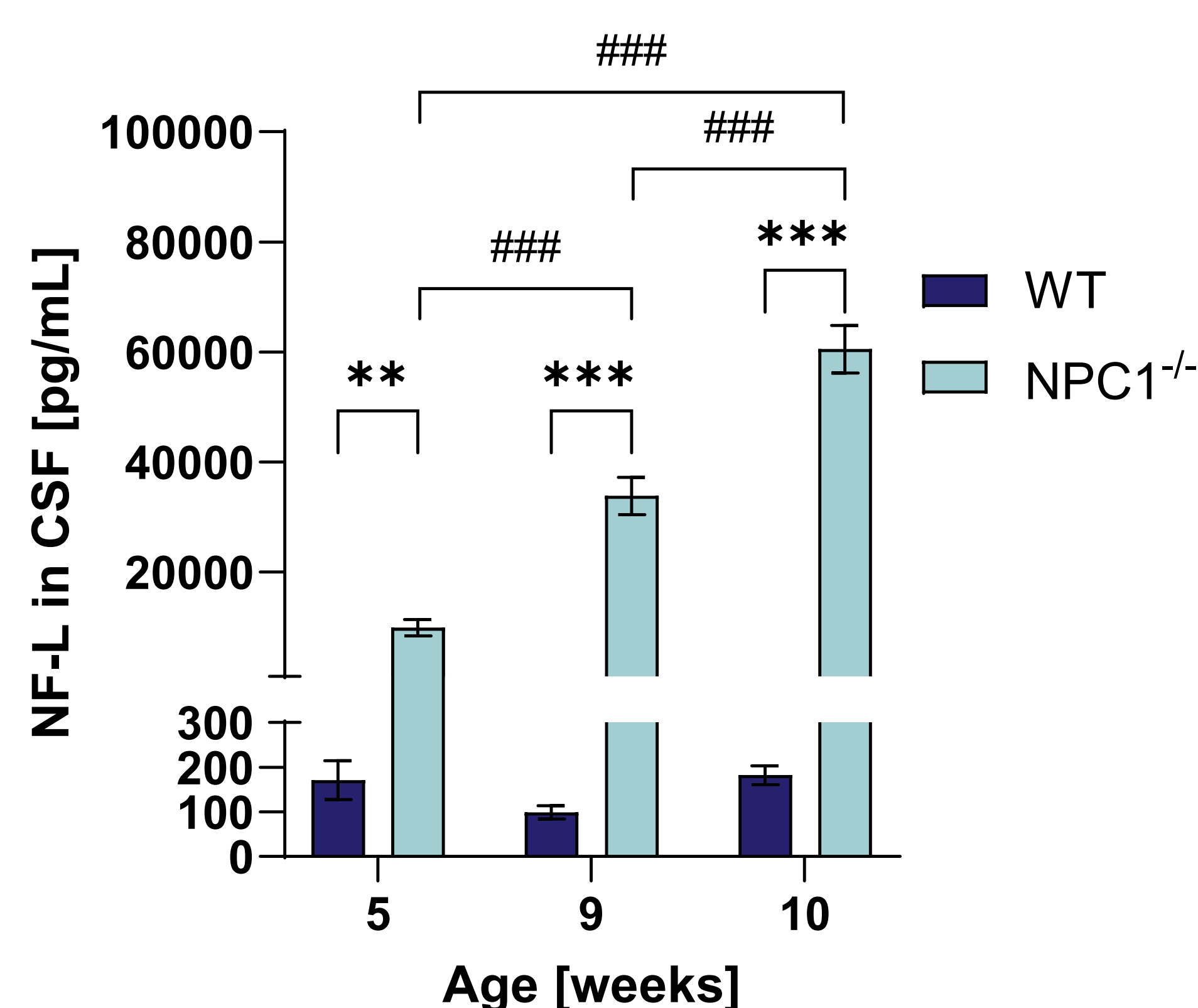


▲ **Figure 1: Representative images of Evans Blue distribution after ICV injection into P0-P3 pups.** Directly after injection, brains were macroscopically investigated for Evans Blue distribution. Brains of P0-P3 pups were investigated as a whole or cut with brain matrix to visualize the internal distribution of the dye.

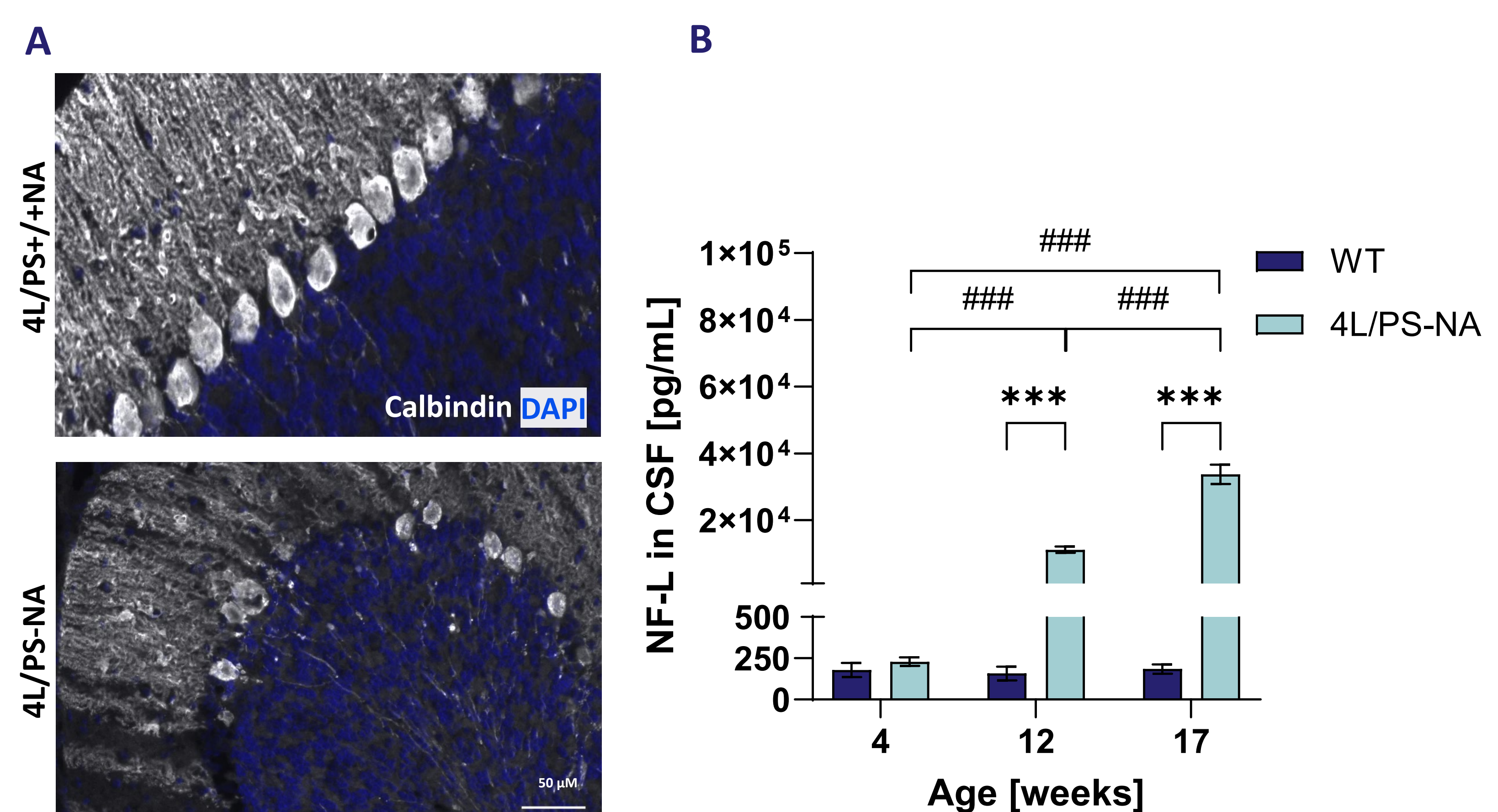
► **Figure 2: Representative images of sagittal brain sections after ICV injection of AAV9-GFP at 0.24 mm from bregma (A), 1.44 mm from bregma (B) and 3.12 mm from bregma (C).** GFP expression was visualized by immunofluorescence staining with Alexa Fluor 488-conjugated anti-GFP antibody. Nuclei were counterstained with DAPI. Cut-outs highlight GFP+ cells. Cb: cerebellum; CP: choroid plexus; Cx: cortex; DAPI: 4',6-diamidino-2-phenylindole; GFP: green fluorescent protein.



Early Neuronal Loss in LSD Mouse Models Monitored via NF-L Level in Longitudinal CSF Samples



▲ **Figure 3: Quantification of NF-L levels in the CSF of NPC1^{-/-} mice and wild type littermates as marker for neuronal loss.** CSF samples were repeatedly taken from the same animals at the age of 5, 9, and 10 weeks. Two-way ANOVA followed by Šidák's multiple comparison *post hoc* test; n = 7-8 per group; Mean ± SEM; **p < 0.01; ***/###p < 0.001.



▲ **Figure 4: Degeneration of cerebellar Purkinje cells (A).** Neuronal loss is also described in the 4L/PS-NA mouse model of Gaucher disease, especially in the cerebellum. Representative images of calbindin and DAPI immunolabeling in 17 weeks old 4L/PS-NA and 4L/PS-+/NA mice. **Longitudinal quantification of NF-L levels in the CSF of 4L/PS-NA mice and wild type littermates (B).** CSF samples were repeatedly taken from the same animals at the age of 4, 12 and 17 weeks. Two-way ANOVA followed by Šidák's multiple comparison *post hoc* test. n = 13 per group; Mean ± SEM; ***/###p < 0.001.

Meet Scantox at booth #106

For more information about the models please visit: www.scantox.com

Scantox Neuro GmbH, Parkring 12, 8074 Grambach, Austria