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Supplemental Information

Neurotropic Properties of AAV-PHP.B Are Shared among Diverse Inbred Strains of Mice

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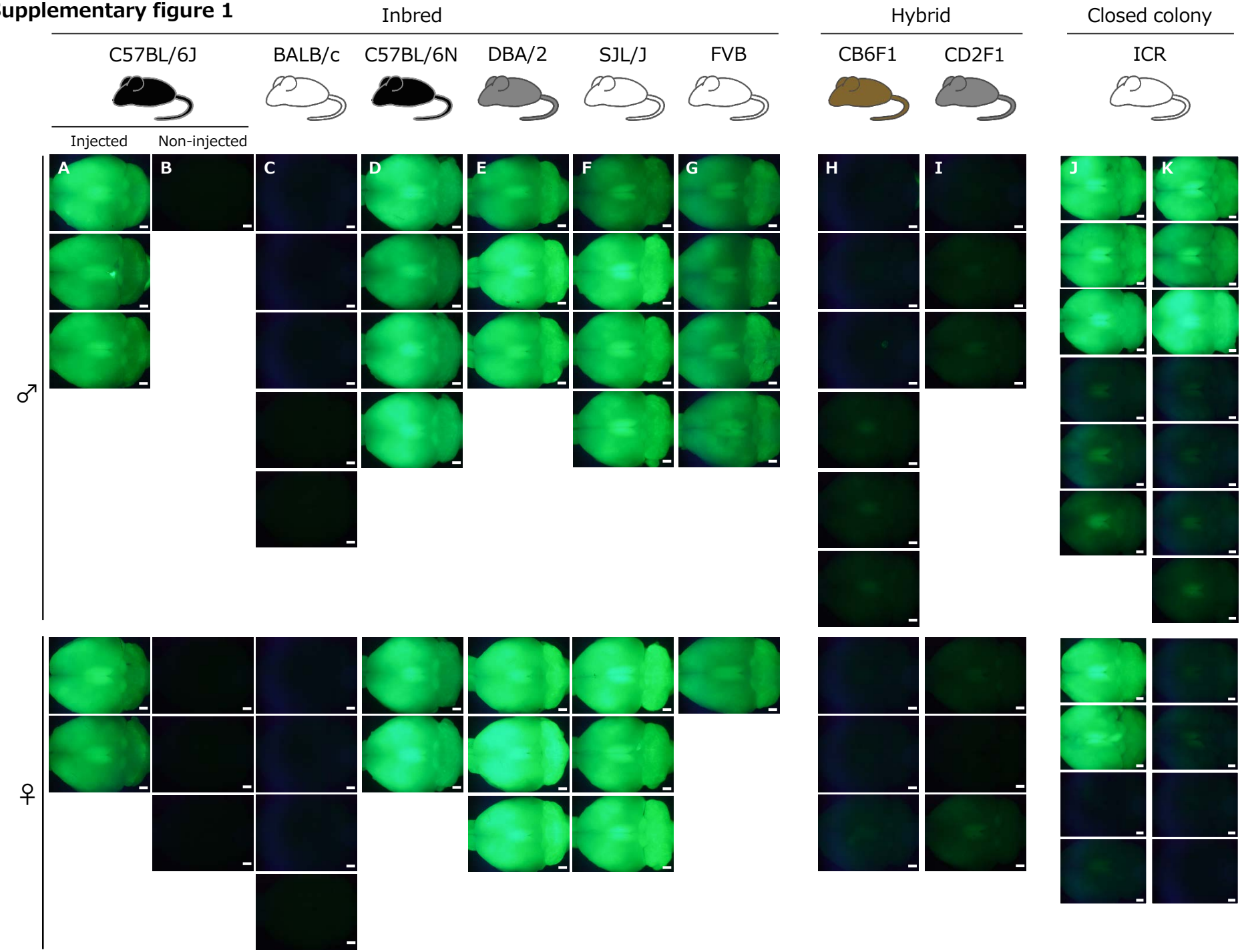
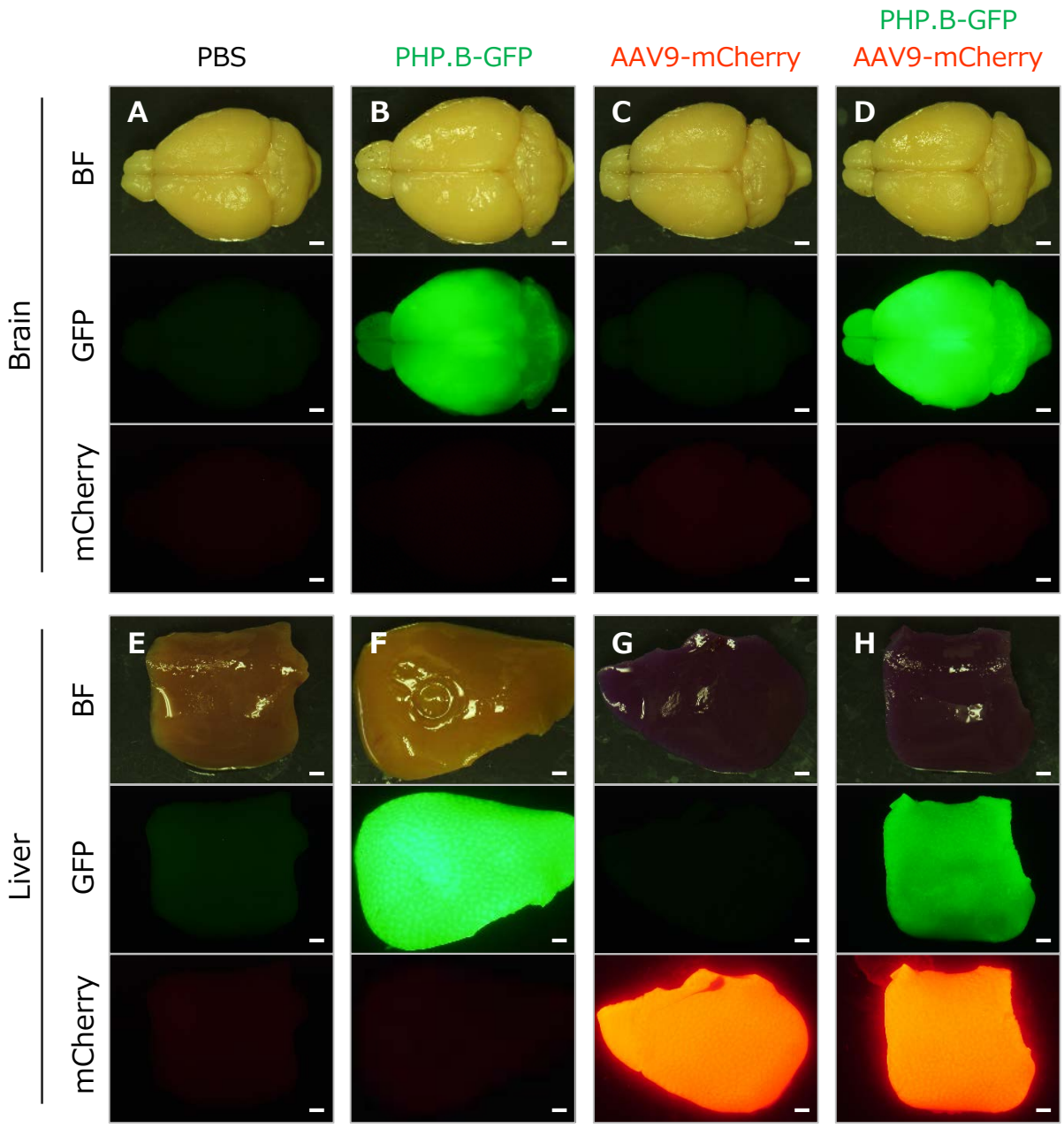
Supplementary figure 1



Supplementary figure 1. GFP fluorescence images of whole brains from various inbred, hybrid and outbred mouse strains that received intravenous injection of PHP.B expressing GFP (A, C-K) and those from non-injected control C57BL/6J mice (B). Details of mice and viral titers are presented in Table 1. Upper and lower images were obtained from male and female mice, respectively. The hair color of each mouse strain is displayed as an illustration above each brain image. Scale bars: 1 mm.

Supplementary figure 2



Supplementary figure 2. Selective penetration of PHP.B after intravenous infusion of the mixture of PHP.B and AAV9. C57BL/6J mice received intravenous infusion of PBS, PHP.B expressing GFP (PHP.B-GFP), AAV9 vectors expressing mCherry (AAV9-mCherry) or a mixture of the 2 viruses. Two weeks after the injection, fluorescent protein expression in the brains and livers was examined. Upper, middle and lower panels show bright field (BF), GFP, and mCherry images, respectively, from brains (A-D) and livers (E-H). Note the absence of AAV9-mediated mCherry expression in the brain after co-administration of AAV9 with PHP.B, in contrast to robust expression of both GFP and mCherry in the liver (right panels). Scale bars: 1 mm.

Supplementary materials and methods

1. Animals

All animal procedures were performed according to the institutional and national guidelines and were approved by the Institutional Committee of the Gunma University (No. 18-062 and 18-019). C57BL/6J, C57BL/6N, BALB/c, DBA/2, CB6F1, CD2F1, and ICR mice were purchased from Japan SLC (Hamamatsu, Japan), FVB/N mice were from CLEA Japan (Tokyo, Japan) and SJL/J mice were from Charles River Laboratories Japan (Yokohama, Japan). C57BL/6J, FVB/N, and SJL/J mice were bred at the laboratory of Neurophysiology and Neural Repair or the Gunma University Bioresource Center. We used following numbers of mice in this experiment: seventeen C57BL/6J (including 3 non-injected control mice and 8 mice for the experiment of double AAV vector injection), twelve BALB/c, six C57BL/6N, six DBA/2, seven SJL/J, five FVB/N, six CB6F1, six CD2F1, and 25 ICR mice (Table 1). The mice were maintained on a 12-h light/dark cycle and had free access to food and water.

2. Construction and production of AAV-PHP.B

AAV-PHP.B was produced using an expression plasmid pAAV/CBh-EGFP-WPRE and a packaging plasmid pAAV-PHP.B as described previously [1]. To obtain pAAV/CBh-EGFP-WPRE, the constitutive CBh promoter was inserted to the pAAV plasmid at XhoI and AgeI restriction enzyme sites upstream the *EGFP* gene. pAAV-PHP.B was constructed from the pAAV2/9 plasmid [2], which was kindly provided by Dr. J. Wilson.

Recombinant single-strand AAV-PHP.B vectors were produced using HEK293T cells (HCL4517; Thermo Fisher Scientific; Waltham, MA, USA), as described previously [1, 3]. Briefly, HEK293T cells, which were cultured in Dulbecco's Modified Eagle Medium (D-MEM; D5796-500Ml, Sigma-Aldrich, St Louis, MO, USA) supplemented with 8% fetal bovine serum (Sigma-Aldrich), were transfected with three plasmids: pAAV/CBh-EGFP-WPRE, pHelper (Stratagene, La Jolla, CA), and pAAV-PHP.B, using Polyethylenimine. Viral particles were harvested from the cultured medium 6 days after transfection and were concentrated by precipitation with 8% polyethylene glycol 8000 (Sigma-Aldrich) and 500 mM

sodium chloride. The precipitated AAV-PHP.B particles were re-suspended in D-PBS and purified with iodixanol (OptiPrep; Axis-Shield Diagnostics, Dundee, Scotland) continuous gradient centrifugation. The viral solution was further concentrated in D-PBS using Vivaspin 20 (100,000 MWCO PES, Sartorius, Gottingen, Germany). The genomic titers of the viral vector were determined by real-time quantitative PCR using Power SYBR Green PCR Master Mix (Thermo Fisher Scientific), using the primers 5'-CTGTTGGGCACTGACAATTC-3' and 5'-GAAGGGACGTAGCAGAAGGA-3', which targeted the WPRE sequence. The expression plasmid was used as a standard. We adjusted the viral titer to 1.0×10^{12} vector genome (vg)/ml.

3. Intravenous injection and measurement of GFP fluorescence intensity from whole brains

For intravenous injection, we deeply anesthetized mice with a combination of ketamine and xylazine. One hundred μl of AAV-PHP.B (1.0×10^{12} vg/ml) was injected into the orbital sinus using a 0.5 ml syringe with a 30-gauge needle (08277; Nipro, Osaka, Japan).

Fourteen to 17 days after viral injection, the mice were deeply anesthetized with a mixture of ketamine and xylazine, and transcardially perfused with 1 × PBS (-) and fixed by 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB). Subsequently, the brains were incubated in 4% PFA solution for 6 to 8 hours at 4 °C. Bright field and native GFP fluorescence images of the whole brains were acquired using a fluorescence stereoscopic microscope (VB-7010; Keyence, Osaka, Japan). Subsequently, the brains were cut into 50-μm-thick sections using a Vibrating Blade Microtome (VT1200S; Leica, Wetzlar, Germany). For measuring GFP fluorescence intensity of whole brains, the margin of the whole brain was traced on the bright field image and the GFP fluorescence intensity in the enclosed area was measured using ImageJ (Fiji). The background intensity (autofluorescence) of the brain was obtained from three non-injected C57BL/6J mouse brains. The averaged background intensity was subtracted from GFP fluorescence values of all samples. Finally, the values were normalized to the mean value (1.0) from C57BL/6J mouse brains.

4. Immunohistochemistry

Brains were cut into 50-μm-thick sections using a Vibrating Blade Microtome (VT1200S; Leica, Wetzlar, Germany). Briefly the free floating sections were permeabilized and blocked with blocking solution containing 0.1 M PB, 5-fold-diluted G-Block (GenoStaff, Tokyo, Japan), 0.5% (w/v) Triton X-100, and 0.025% NaN_3. The sections were then incubated in blocking solution containing the following antibody overnight at room temperature (24 to 26 °C): rat monoclonal anti-GFP (1:1,000; 04404-84; Nacalai tesque, Kyoto, Japan), mouse monoclonal anti-NeuN (1:1,000; MAB377; Merck, USA). For visualization of the bound primary antibodies, the sections were incubated for 4 hours at room temperature in the blocking solution containing the following secondary antibodies: Alexa Fluor 488-conjugated donkey anti-rat IgG (1:1,000; A21208; Thermo Fisher Scientific), Alexa Fluor Plus 555-conjugated goat anti-mouse IgG (1:2,000; A10037; Thermo Fisher Scientific). The immunostained sections were mounted using the ProLong Diamond antifade reagent (P36961; Thermo Fisher Scientific). Fluorescent images were acquired using fluorescence microscope (BZ-X700, Keyence).

5. Statistical analyses

We used GraphPad PRISM version 6 (GraphPad Software, San Diego, CA, USA) for statistical analysis and output of graphic images. Statistical differences were analyzed using one-way ANOVA with Dunnett's multiple comparison tests or Kruskal-Wallis with Dunn's multiple comparison test. GFP fluorescence intensity of whole brains from different mouse strains were compared with those from BALB/c (asterisk) and C57BL/6J (dagger) in each figure. The data were expressed as the mean ± standard error of the mean.

References

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3. Nitta, K., Matsuzaki, Y., Konno, A., and Hirai, H. (2017). Minimal Purkinje cell-specific PCP2/L7 promoter virally available for rodents and non-human primates. Mol. Ther. Methods Clin. Dev. *6*, 159–170.