Supplementary Materials for

Apolipoprotein E, low-density lipoprotein receptor, and immune cells control blood-brain barrier penetration by AAV-PHP.eB in mice

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### A

|       | Striatum | Cortex1 | CA1 | CA2 | Dentate gyrus | Thalamus1 |
|-------|----------|---------|-----|-----|---------------|-----------|
| C57BL/6 wild-type | ![Image](image1) | ![Image](image2) | ![Image](image3) | ![Image](image4) | ![Image](image5) | ![Image](image6) |
| Apoe<sup>−/−</sup> | ![Image](image7) | ![Image](image8) | ![Image](image9) | ![Image](image10) | ![Image](image11) | ![Image](image12) |
| Ldr<sup>−/−</sup> | ![Image](image13) | ![Image](image14) | ![Image](image15) | ![Image](image16) | ![Image](image17) | ![Image](image18) |

### B

**Roles of ApoE and LDLR in C57BL/6 mouse in AAV-PHP.eB transduction**

![Graph showing fluorescence intensity in different areas of the central nervous system](image)

- **Different areas of the central nervous system:**
  - Striatum
  - Cortex1
  - CA1
  - CA2
  - Dentate gyrus
  - Thalamus1
  - Hypothalamus
  - Midbrain
  - Pons
  - Medulla
  - Cerebellum
  - Spinal cord

- **Statistics:**
  - C57BL/6 wild-type: p = 0.016, p = 0.024
  - Apoe<sup>−/−</sup>: p = 0.035
  - Ldr<sup>−/−</sup>: p = 0.041, p = 0.022
Figure S1 | Transduction of intravenous AAV-PHP.eB into various brain regions of C57BL/6 wild-type, Apoe<sup>−/−</sup> and Ldlr<sup>−/−</sup> mice. (A) Representative fluorescent images of the indicated tissues in the indicated mice following intravenous administration of AAV-PHP.eB. The blue fluorescence indicates Hoechst nuclear staining. (B) Quantification of the red fluorescence intensity in the indicated mice and brain regions 3 weeks after the AAV-PHP.eB administration (n = 4 for each group). For the comparison of C57BL/6 wild-type with either Apoe<sup>−/−</sup> or Ldlr<sup>−/−</sup> mice, p values were determined by Tukey post-hoc test (medulla) or Games-Howell post-hoc test (all other regions). Data are mean ± s.e.m.
Figure S2 | Brain and liver transduction of AAV-PHP.eB and AAV-9 and effect of AAV-PHP.eB on the permeability of the blood-brain barrier in mice. (A)

Representative images of the AAV-PHP.eB and AAV-9 transduction in the indicated tissues in the indicated mice. While transducing to both the brain and the liver (red fluorescence) in wild-type C57BL/6 mice, AAV-PHP.eB is able to transduce only the
liver cells in *Apoe<sup>−/−</sup>* or *Ldlr<sup>−/−</sup>* mice (n = 3 for each group). In contrast, AAV-9 transduces the liver cells, but fails to transduce brain cells in all three mouse genotypes (n = 3 for each group). **(B)** Evaluation on the permeability of the blood-brain barrier after the AAV-PHP.eB injection. Intravenously injected AAV-PHP.eB does not significantly increase the barrier permeability, measured by Evans Blue infiltration, in C57BL/6 mice (n = 4 for each group).
Figure S3 | Effect of plasma on the central nervous system transduction of intravenous AAV-PHP.eB in Apoe<sup>−/−</sup> mice. (A) Schematic showing plasma isolation from wild-type mouse blood and preparation of AAV-PHP.eB expressing the mScarlet gene mixed with plasma. Thirty minutes after being mixed with ApoE-containing plasma prepared from either C57BL/6 or BALB/c mice, AAV-PHP.eB was administered intravenously to Apoe<sup>−/−</sup> mice. (B) Representative images showing transduction in the medulla regions 3 weeks after systemic delivery of AAV-PHP.eB.
plus plasma. Images from an $Apoe^{-/-}$ mouse which was not treated with plasma are
shown as a negative control. (C) Quantification of the fluorescence intensity of the
indicated brain regions. The p values were determined by one-way ANOVA. The
means ± s.e.m are indicated (n = 4 for each group).
Figure S4 | Comparison of the local transduction of AAV-PHP.eB in the brains in *Apoe<sup>−/−</sup>* and *Ldlr<sup>−/−</sup>* mice. Representative fluorescent images (red, A and D) of the hippocampi (B and E) in *Apoe<sup>−/−</sup>* (A-C) and *Ldlr<sup>−/−</sup>* (D-F) mice following the stereotactic microinjection of AAV-PHP.eB into the dentate gyrus. A and B are merged in C, and D and E in F. The blue fluorescence indicates Hoechst nuclear staining. n = 2 for each group.
Figure S5 | Transduction of intravenous AAV-PHP.eB to the brain of in C.B-17 SCID mice lacking both T and B cells. (A) Representative images of the indicated tissues, 3 weeks after the intravenous AAV-PHP.eB administration to BALB/c wild-type and C.B-17 SCID mice. The blue fluorescence indicates Hoechst nuclear staining. magn: magnification. (B) Analyses of the AAV-PHP.eB transduction in the indicated areas of BALB/c (n = 3) and C.B-17 SCID mice (n = 4), 3 weeks after the AAV-PHP.eB injection; the p value was determined by two-tailed Student's t-test. Data are mean ± s.e.m.
Figure S6 | Lymphocyte and B cell populations in the spleens of ldlr<sup>-/-</sup> and ldlr<sup>+/+</sup> mice. (A-C) Flow cytometry analyses of lymphocytes in the spleen (A) and of B cells in ldlr<sup>-/-</sup> (B) and ldlr<sup>+/+</sup> (C) mice. (D) Quantification of the ratios of CD3<sup>-</sup>B220<sup>+</sup> B cells to CD45<sup>+</sup> lymphocytes. The means ± s.e.m are indicated (n = 4 for each group). The p values > 0.05 (two-tailed Student's t-test).