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Supplemental information

Optimal different adeno-associated virus capsid/promoter combinations to target specific cell types in the common marmoset cerebral cortex

Yasunori Matsuzaki, Yuuki Fukai, Ayumu Konno, and Hirokazu Hirai

Figure S1

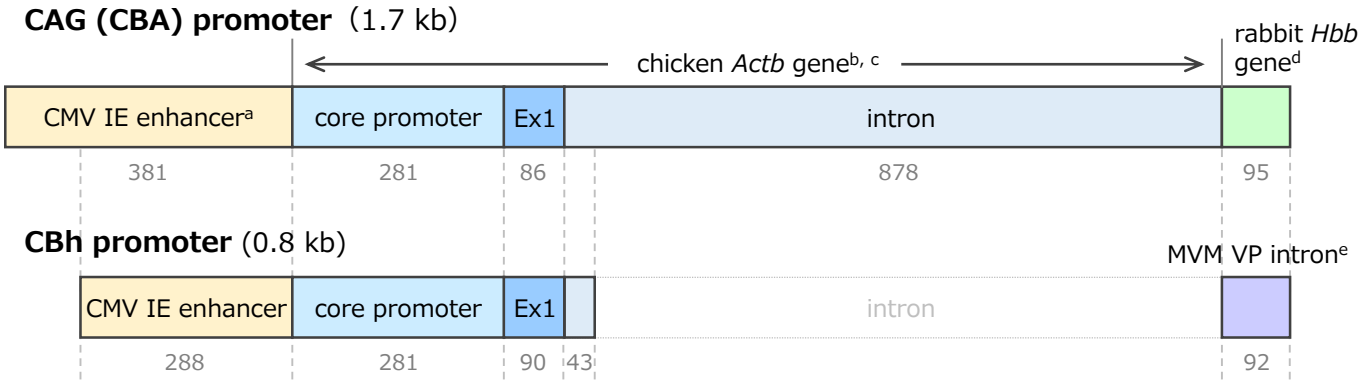

Figure S1. The structural comparison of CAG promoter and CBh promoter which were used in this study. The CMV Immediate Early (IE) enhancer was shown in yellow, sequences related to the chicken β-actin (*Actb*) gene including core promoter region, exon 1 (Ex1) and intron, were shown in blue colors, the rabbit β-globin (*Hbb*) gene sequence was shown in green, and the sequence of minute virus of mouse (MVM) was shown in purple. The homologous regions of each promoter were indicated by gray dotted lines, and the length of each component was indicated by gray numbers. There seemed to be a 5-base spacer sequence between the *Actb* intron and the MVM VP intron in the CBh promoter, but the notation was omitted. There are insertions, deletions, and mutations in the CMV IE enhancer and *Actb* gene between each promoter but omitted from the notation. The following DNA databases were used for the structural mapping of both promoters; [a]Genbank accession no.: MN920393.1, [b]Genbank accession no.: X00182.1, [c]Ensemble accession no.: ENSGALT00010051464.1, [d]Genbank accession no.: M18818.1, [e]Genbank accession no.: NC_001510.1.

Figure S2

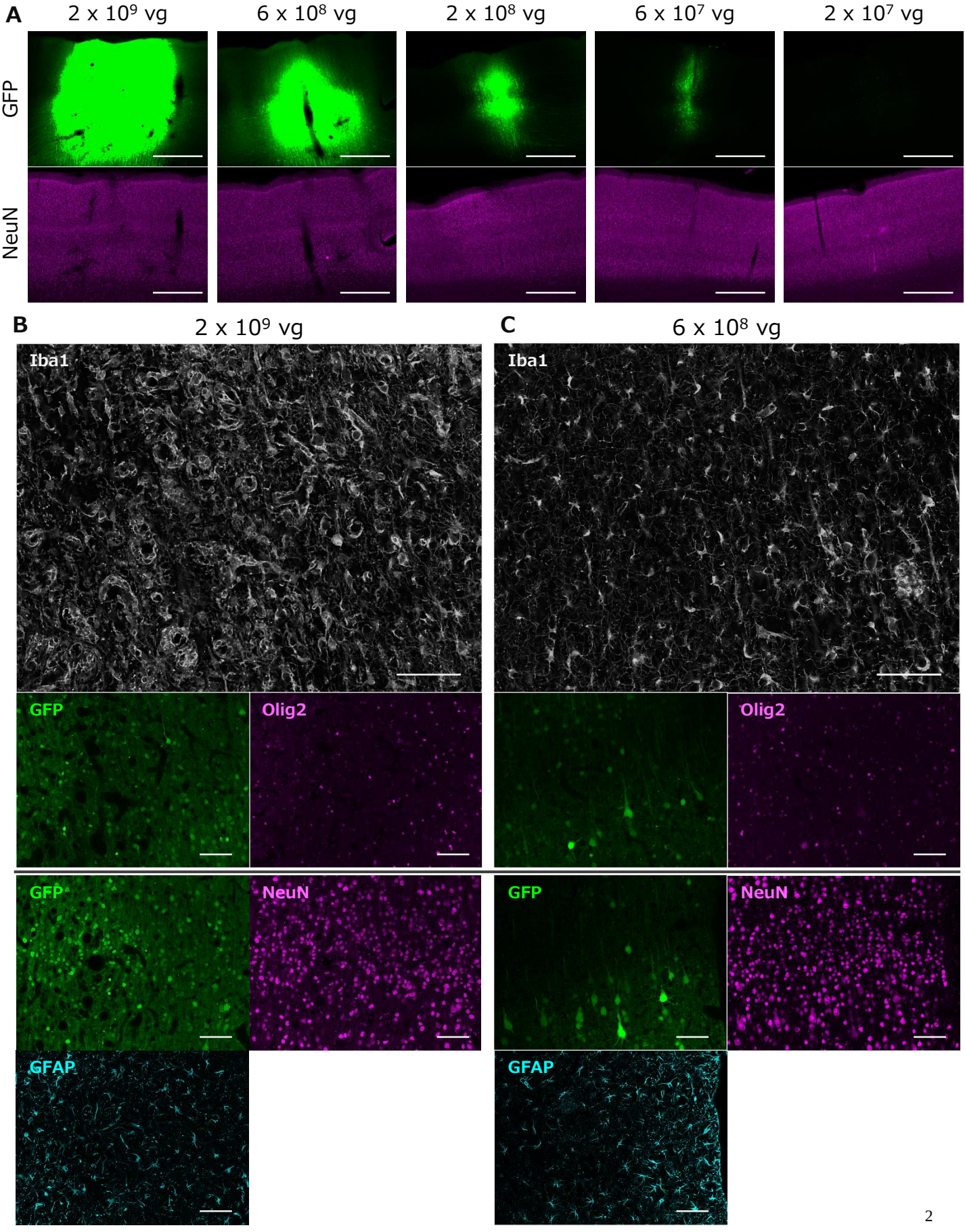
A
2 x 10⁹ vg
6 x 10⁸ vg
2 x 10⁸ vg
6 x 10⁷ vg
2 x 10⁷ vg
GFP
NeuN
B
2 x 10⁹ vg
C
6 x 10⁸ vg
Iba1
Iba1
GFP
Olig2
Olig2
GFP
NeuN
GFP
NeuN
GFAP
GFAP

Figure S2. Spread of AAV vectors and microglial activation by direct injection of AAV vectors into the marmoset cerebral cortex. **(A)** 3-fold dilution series of AAV2 vectors expressing EGFP by the CBh promoter was injected into the marmoset cerebral cortex. Immunofluorescent images of EGFP (upper) and NeuN (lower) in the marmoset cortex four weeks after AAV injection at the doses indicated. Increasing the amount of virus injected expanded the area of EGFP expression. However, no obvious neuronal loss was observed even when the highest dose was injected. **(B-C)** Injection of AAV2 at 2.0×10^9 vg caused considerable activation of microglia **(B)**, while no apparent activation was observed by a lower amount of AAV2 at 6.0×10^8 vg or lower **(C)**. The top two large images show microglia immunolabeled for Iba1. The smaller images below are immunohistochemistry for GFP, Olig2, NeuN, and GFAP, as labeled on the top left of each image. The images above and below the gray line in the center were obtained using different tissue sections. Scale bar, 100 μm.

Figure S3

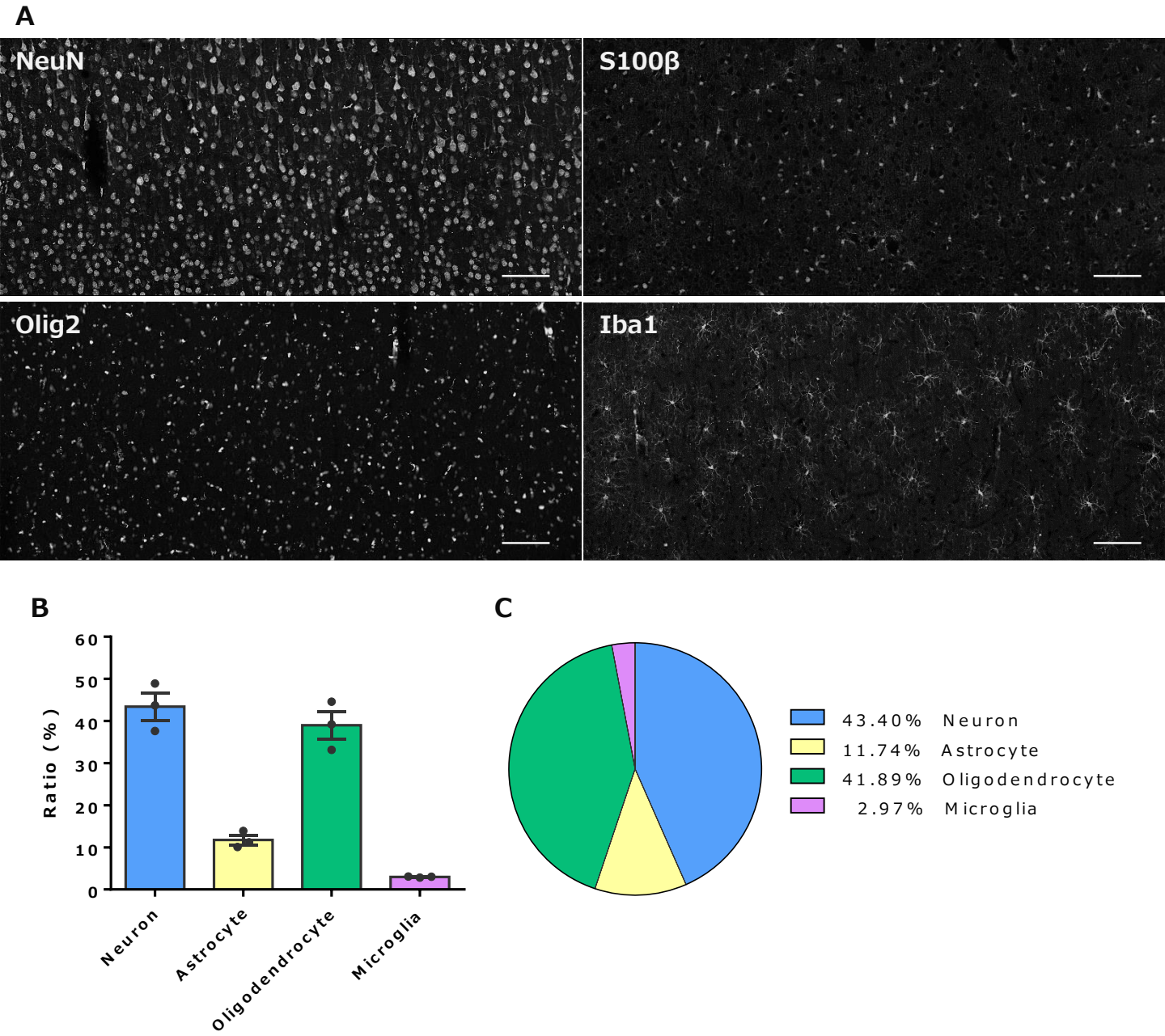

Figure S3. The proportions of different cell types endogenously present in the marmoset cerebral cortex were assessed immunohistochemically. (**A**) Representative fluorescent images immunolabeled for NeuN, S100β, Olig2, and Iba1. Scale bar, 100 μm. (**B**, **C**) Summary bar graph (**B**) and pie chart (**C**) showing the percentages of neurons, astrocytes, oligodendrocytes, and microglia. Error bars indicate S.E.M., and dots in the graph indicate the respective values for each of the individual marmosets.

Figure S4

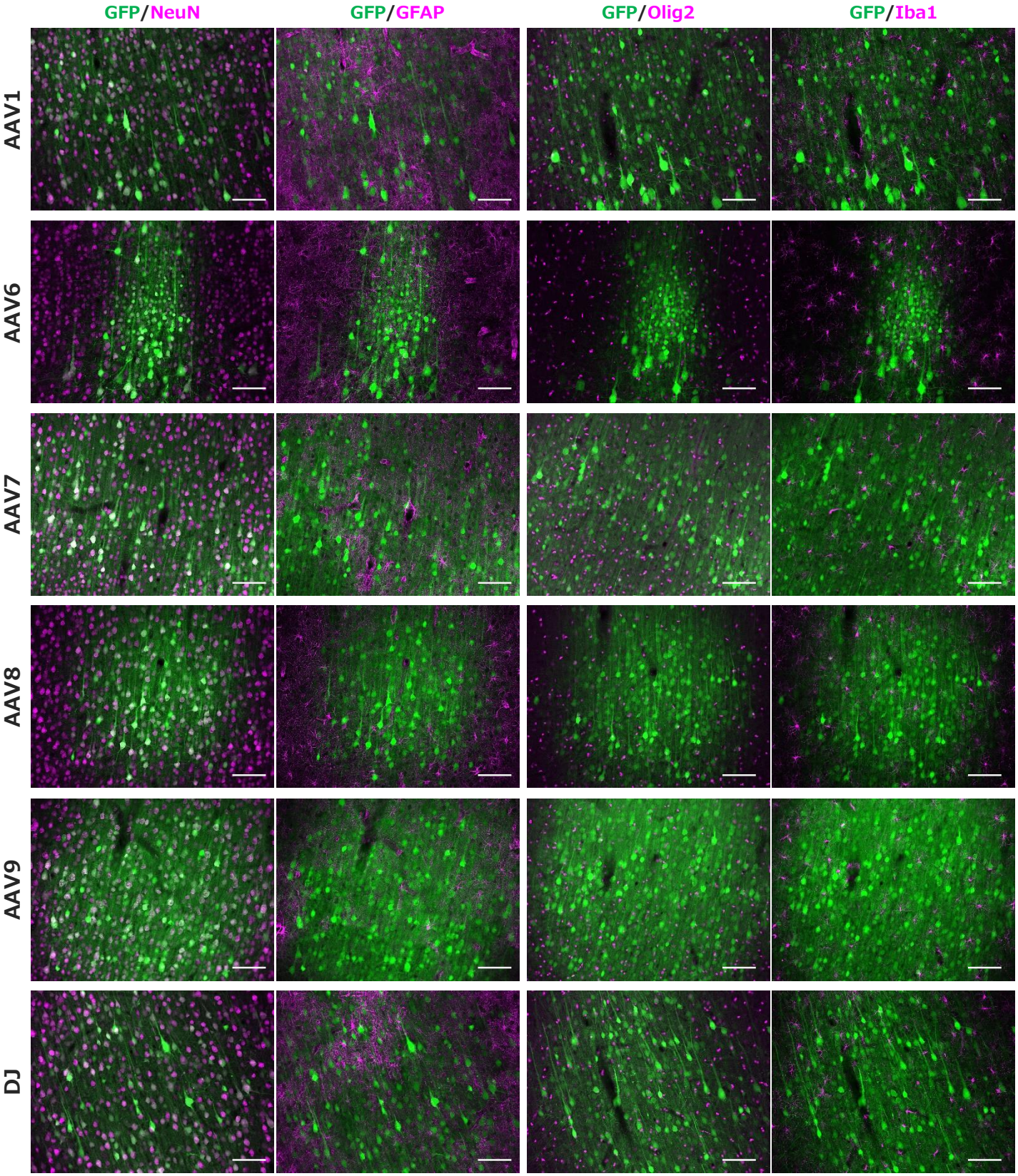

Figure S4. Representative immunohistochemical images of cerebral cortexes where were injected AAV1, 6, 7, 8, 9 and DJ. All images show GFP (green) and co-immunostained images with NeuN, GFAP, Olig2, and Iba1 (magenta) as counterstains, respectively. Scale bar, 100 μm.

Figure S5

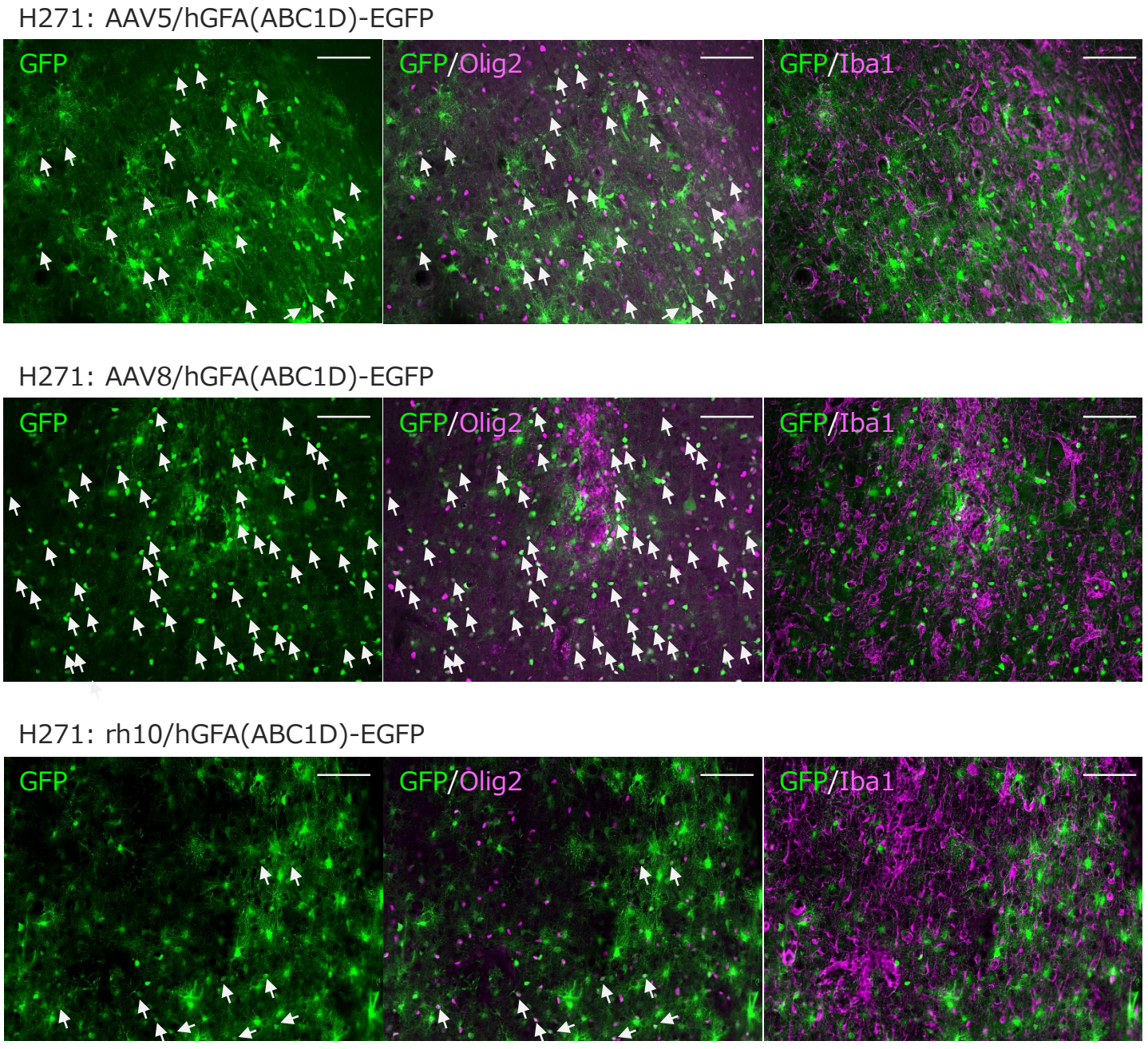

Figure S5. Examples of extensive oligodendrocyte transduction by the astrocyte-specific hGFA(ABC1D) promoter in the case of some AAV capsids. Immunofluorescent images of the cerebral cortex from a marmoset (ID: H271, see Table 1) 4 weeks after injection of AAV5 (top), AAV8 (middle), and AAVrh10 (bottom) expressing EGFP by the astrocyte-specific hGFA(ABC1D) promoter. Cerebellar sections were immunostained in triplicate for EGFP, Olig2, and Iba1. Numerous GFP-expressing cells were co-immunolabeled for Olig2 (arrows) when AAV5 and AAV8 were used, indicating extensive transduction of oligodendrocytes despite employing the astrocyte-specific promoter. Scale bar, 100 μm.