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Supplemental Information

GluA4-Targeted AAV Vectors Deliver Genes Selectively to Interneurons while Relying on the AAV Receptor for Entry

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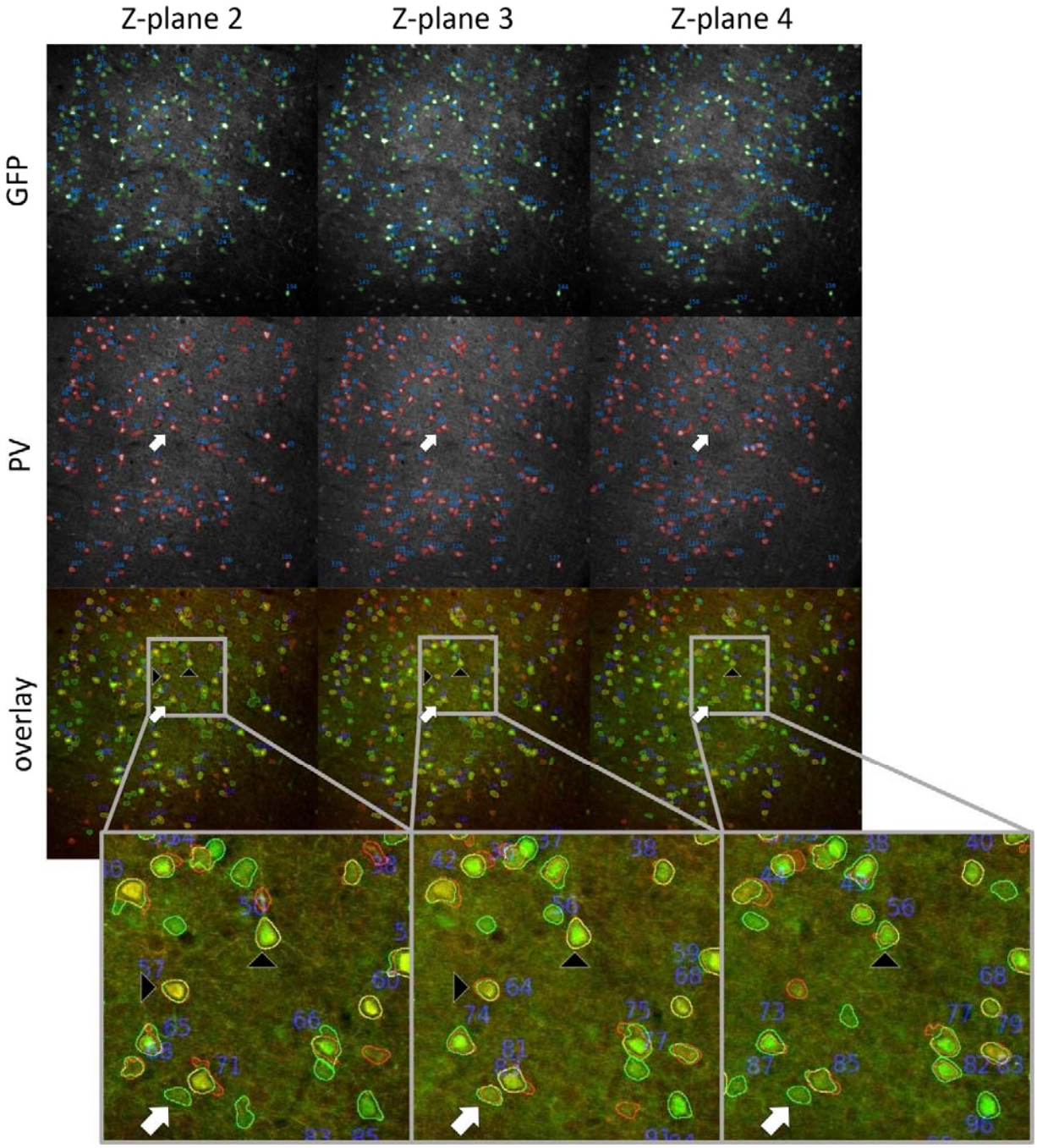

Figure S1: Computer based analysis of brain slices

Representative cell count analysis of one z-stack image via cell profiler software. Three plains of a z-stack of the GFP and PV channel of one representative image of the motor cortex are shown as well as the merge images of both channels. In each channel fluorescently labeled cells were counted via the cell profiler software. Identified cells

were marked with a green (GFP) or red (PV) border and then serially numbered. Only cells labeled with the red and green border and in addition being present in at least two consecutive z-planes images were regarded as truly double positive. Examples for these cells are indicated by black arrow heads in the magnification of the overlay. This way, any software artifacts were excluded from the analysis. Examples of such software artifacts are indicated by the white arrows pointing to PV+ cells present in z-plane 3 but not in z-planes 2 or 4.

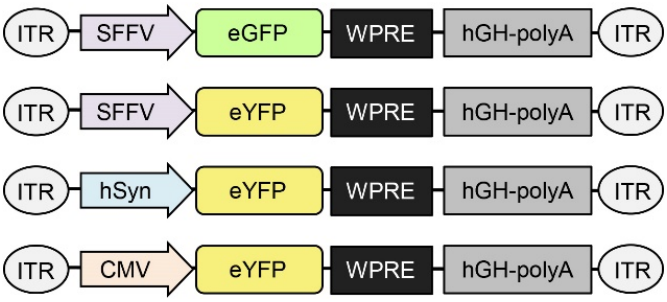

Figure S2: Transgene constructs for AAV vector preparation

Schematic drawing of the transgene cassette flanked by two inverted terminal repeats (ITR) applied for the generation of AAV vectors. The promoter of the spleen focus-forming virus (SFFV), human Synapsin (hSyn) or Cytomegalovirus (CMV) was used to drive the expression of the enhanced green fluorescent protein (eGFP) or the enhanced yellow fluorescent protein (eYFP). The transgene is followed by the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) and a polyadenylation signal from human growth hormone (hGH-polyA).

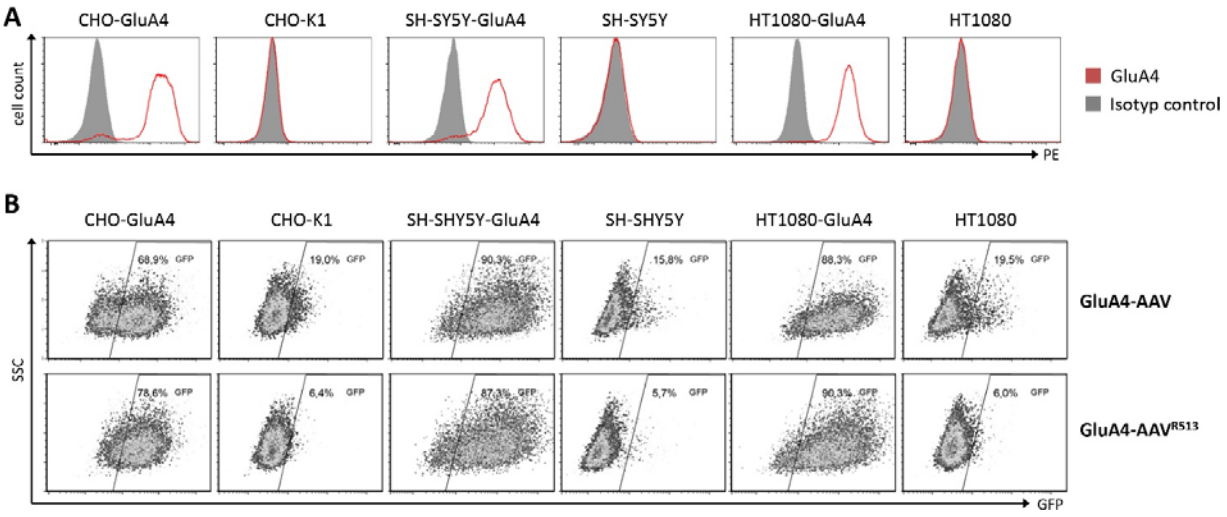

Figure S3: Characterization and transduction of GluA4 expressing cell lines

(A) GluA4 cell surface expression. The indicated cell lines were stably transduced with a lentiviral vector encoding the myc-tagged GluA4 receptor. Parental cell lines and cell lines expressing GluA4 were stained with a PE conjugated anti-myc antibody for flow cytometry analysis. Representative flow cytometry histograms are shown. **(B)** The indicated GluA4-positive and -negative cell lines were incubated with GluA4-AAV or GluA4-AAV$_{mut}^{R513A}$ at a GOI of 7.9x10^5 (CHO) or 2.6x10^5 (SH-SY5Y and HT1080), respectively. Cells were analyzed 72 hr after transduction by flow cytometry. Representative dot plots are shown.

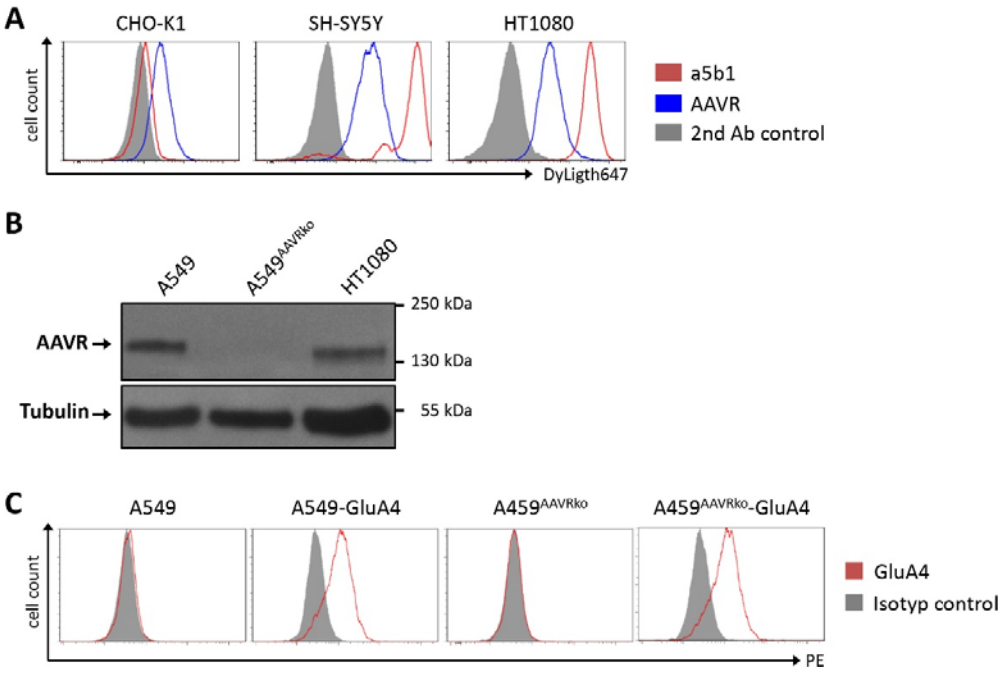

Figure S4: Expression of integrin α5β1, AAVR and GluA4 on various cell lines

(A) Detection of integrin α5β1 and AAVR on the surface of the indicated cell lines. The cells were incubated with antibodies specific for human integrin α5 or AAVR in combination with a DyLigth647 conjugated secondary antibody before flow cytometry analysis. Representative flow cytometry histograms are shown. **(B)** Detection of AAVR in cell lysates. Cell lysates of indicated cells lines were separated on a SDS-polyacrylamid gel by electrophoresis. After transferring the proteins to a PVDF membrane, AAVR was detected using a mouse anti-AAVR antibody (1:500) in combination with HRP conjugated goat anti-mouse antibody (1:2000). Tubulin was stained as loading control using a rabbit anti-tubulin (1:1000) and goat anti-rabbit-HRP (1:2000) antibody. **(C)** GluA4 cell surface expression. The indicated cell lines were stably transduced with a lentiviral vector encoding the myc-tagged GluA4 receptor. Parental cell lines and cell lines expressing GluA4 were stained with a PE conjugated anti-myc antibody for flow cytometry analysis. Representative flow cytometry histograms are shown.

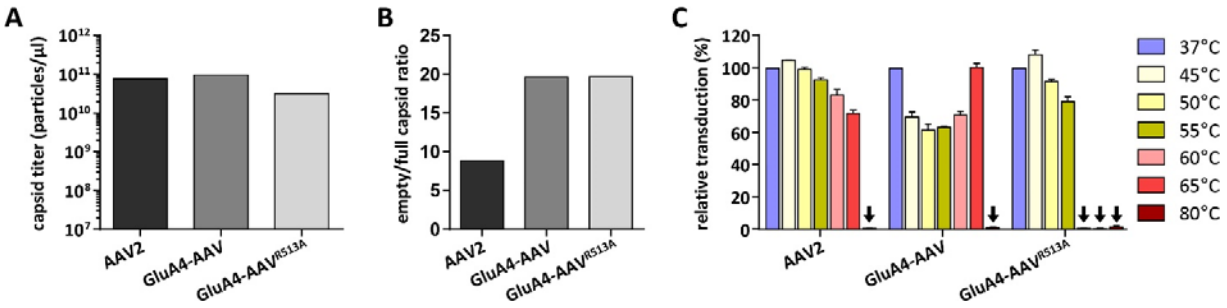

Figure S5: Biophysical properties of GluA4-AAV^{513A}

(A) Capsid titers of the indicated vector types determined by A20-specific antibody ELISA. **(B)** Ratio of empty and genome packaged (full) particles. The capsid titers determined by ELISA were divided by genomic titers as determined by qPCR with primers specific for the ITRs. **(C)** Thermostability of AAV vectors. The indicated AAV vectors were incubated for 15 min at the indicated temperature before added to HT1080-GluA4 cells at a GOI of $1.6x10^5$ (GluA4-AAV and GluA4-AAV^{R513A}) or $1x10^4$ (AAV2). Cells were analyzed 72 hr after transduction by flow cytometry detecting GFP. Transduction was normalized to that determined at 37°C for each vector (dark blue), respectively. Each transduction experiment was performed in technical triplicates. The mean and SD are shown.

Supplemental Tables

Table S1: Primer sequences for plasmid construction.

Primer	No.	Sequence (5' to 3')
SFFV MulI for	2298	TATACGCGTGGCAAGCTAGCTGCAGTAACGCC
SFFV BamHI rev	2723	ATACAGGATCCCCCGGGCGACTCAGTC
AAV2 BsiWI for	2195	CTGCACCGTACGTCCTCGGCTCGGCGCATCAA
AAV2 R513A for	2198	AAGTACCACCTCAATGGCGCAGACTCTC
AAV2 R513A rev	2199	GAGAGTCTGCGCCATTGAGGTGGTACTT
AAV2 XcmI rev	2202	CAATCTCCAGACCATGCCTGGAAGAACGCCTTGTGTG

Table S2: Plasmid amounts for AAV productions [ng per cm^2 culture area]

	AAV2	GluA4-AAV	AAV_{mut}	GluA4-AAV^{R531A}	Her2-AAV
Helper plasmid:					
pXX6-80	120	100	120	100	100
Packaging plasmid:					
pRC	40				
pRCVP2koA		33.33	40		33.33
pRCVP2koA-R513A				33.33	
Targeting plasmid:					
pGluA4.2K19-VP2		33.33			
pGluA4.2K19-VP2-R531A				33.33	
pHer2.9.29-VP2					33.33
Transfer plasmid:					
pscAAV-SFFV-GFP	40	33.33		33.33	33.33
pssAAV-SFFV-eYFP or pssAAV-hSyn-eYFP or pssAAV-CMV-eYFP			40		

Supplemental Material and Methods

Determination of capsid titers.

Capsid titers were determined by intact AAV2 particle ELISA. Purified AAV vector stocks were coated and detected with the anti-AAV2 intact particle antibody clone A20 as described previously.[19]

Thermostability test of AAV vectors

Thermostability of AAV vectors was determined by incubating AAV particles at different temperatures before assessing their transduction capability on HT1080-GluA4 cells. For this purpose, $1x10^4$ HT1080-GluA4 cells were seeded into a single well of a 96-well plate. Twenty-four hours later, AAV particles were incubated for 15 min at 37°C, 45°C, 50°C, 55°C, 60°C, 65°C or 80°C before adding to the seeded HT1080-GluA4 cells. Cells were transduced at a GOI of $1.6x10^5$ for GluA4-AAV and GluA4-AAV^{R513A} and a GOI of $1x10^4$ for AAV2. At day three post transduction, transduced cells were determined by flow cytometry analysis based on the indicated percentage of green fluorescent cells. Flow cytometry analysis was performed on the MACSQuant Analyzer 10 (Miltenyi Biotec). Data were analyzed using FCS Express version 4.0 (DeNovo Software).

Detection of surface proteins by flow cytometry

Surface expression of GluA4, integrin α5β1 and AAVR on the respective cell lines was detected by staining $5x10^5$ cells with PE-coupled myc antibody (clone 9B11, Cell Signaling Technology) in case of GluA4 or by incubation with a human α5-specific antibody (clone HA5, Millipore) to detect integrin α5β1 or human KIAA0319L-specific antibody (polyclonal, Abcam) to detect AAVR in combination with a mouse-specific, DyLight649-conjugated secondary antibody (polyclonal, BioLegend). As control, cells were incubated either without or with secondary antibody only. After each staining step, cells were washed twice with washing buffer (PBS, 2% FCS, 0.1% NaN_3). Prior to analysis, cells were fixed with PBS containing 1% formaldehyde. All antibodies were diluted 1:100 in washing buffer. 100 µl diluted antibody were used per $5x10^5$ cells. Primary

antibodies were incubated for 1 h at 4°C and fluorescently labeled antibodies for 30 min at 4°C.

Western Blot

$5x10^5$ cells were lysed in 50 µl RPIA lysis buffer (50 mM Tris/CHl pH 8.0, 140 mM NaCl, 1% NP-40, 0.5% natriumdesoxycholat, 0.1% sodium dodecyl sulfate (SDS)) for 1 h on ice before centrifugation for 10 min at 14000 rpm. The supernatant (cleared cell lysate) were denatured by incubation with 4x SDS sample buffer (4% SDS, 250 mM Tris-HCl, 20% glycerin, 0.03% bromphenol blue, 10% b-mercaptoethanol, pH 8.8) for 10 minutes at 95°C, separated by gel electrophoresis on 10% SDS-polyacrylamid electrophoresis gels, and blotted onto PVDF membranes (GE Healthcare). After blocking with TBS-T (50 mM Tris, 150 mM NaCl, 0.1% Tween-20, pH 7.4) containing 5% horse serum, blots were incubated with mouse anti-KIAA0319L (polyclonal, 1:1,000; Abcam) for detection of AAVR and rabbit anti-tubulin (polyclonal, 1:1,000; CellSignaling) for detection of the housekeeping gene tubulin over night at 4°C. Subsequently, blots were incubated for at least 2 h with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:2,000; Dako). Signals were visualized by ECL Plus Western Blotting Detection System according to the manufacturer (ThermoFisher Scientific). All antibodies were diluted in TBS-T containing 2.5% horse serum.