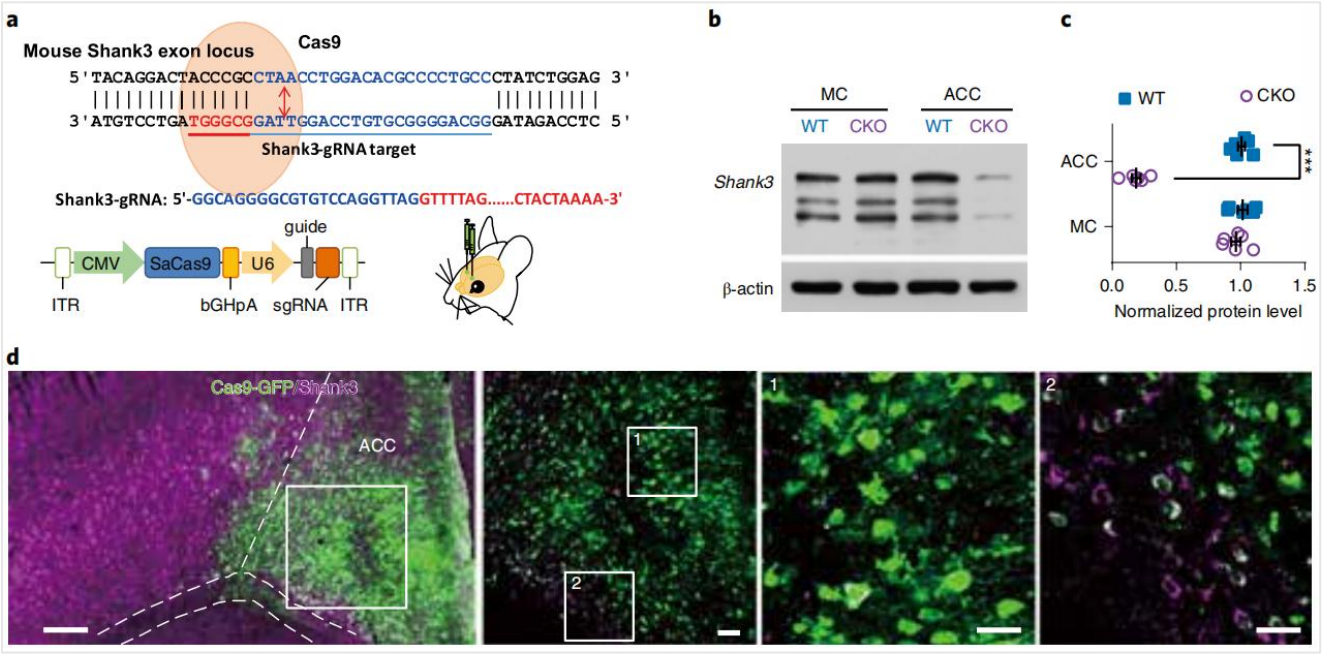
Study Cases of PackGene's AAV Services

Case 1

Products: AAV8 and AAV9 from PackGene Biotech
Injection Method: 300nl was injected bilaterally into the anterior cingulate cortex
Recommended Dose: 50ul, 5×10^11GC/ml
Targeting Site: Anterior Cingulate Cortex
Animal Model: Global Shank3 KO and Conditional ACC Shank3 KO Mouse Model
Journal: Nature Neuroscience, 2022 (IF=28.771)
Paper Title: Anterior cingulate cortex dysfunction underlies social deficits in Shank3 mutant mice
DOI: https://doi.org/10.1038/s41593-019-0445-9

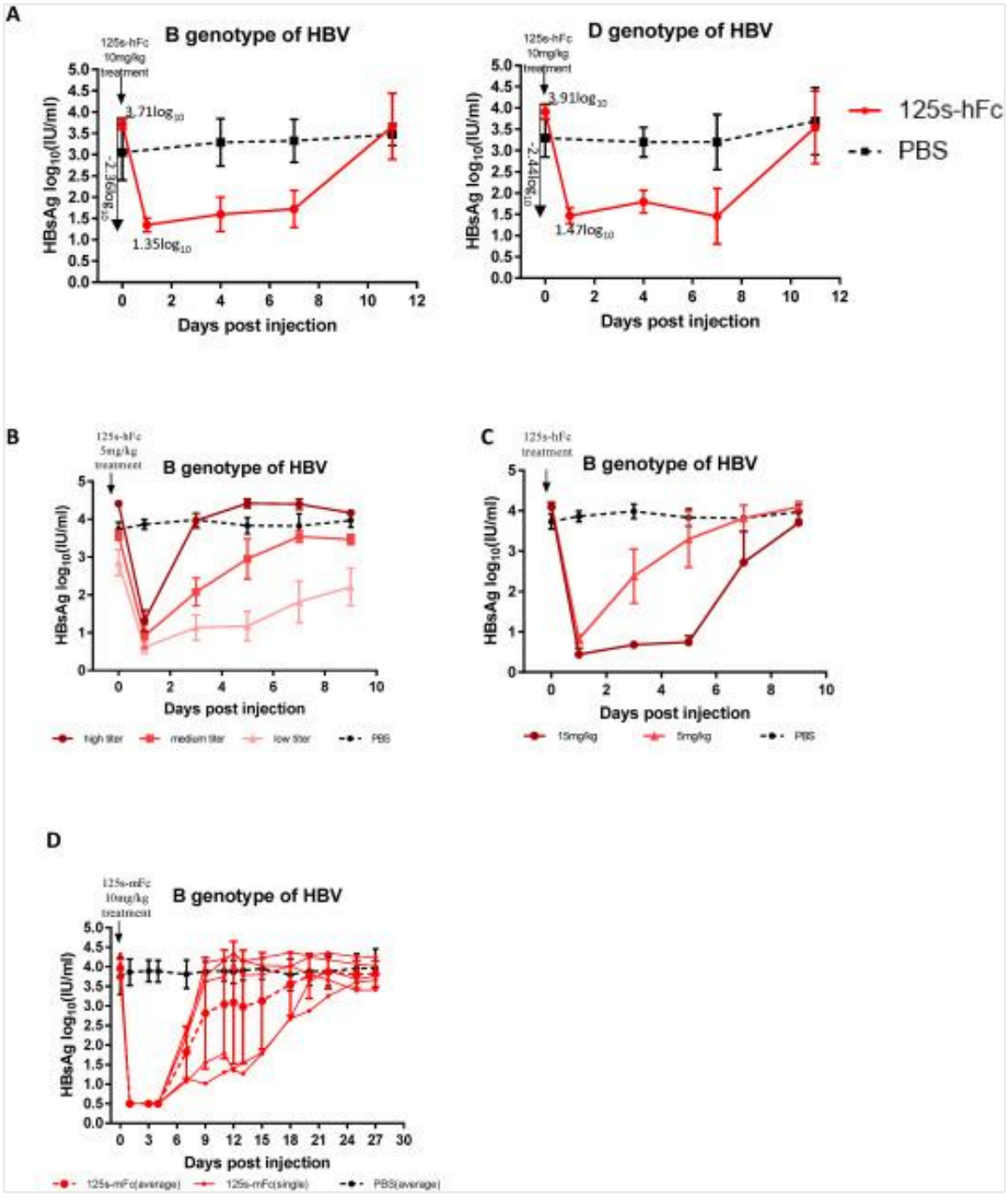


SaCas9 plasmid construction and AAV vector production.

To minimize offtarget effects, three guide RNAs (gRNA1: GGGCTATTCCAGCCTCCCTCC; gRNA2: GGCAGGGGCGTGTCCAGGTTAG; gRNA3: TTGGCGGCCCACACG GGCGCGG) corresponding to SHANK3 were designed using the CRISPR design tool (http://www.rgenome.net/cas-designer/) and cloned into pX601-AAVCMV::NLS-SaCas9-NLS-3xHA-bGHpA;U6::BsaI-sgRNA... After the plasmids were confirmed by sequencing, we transfected those constructs into N2A mouse cells. A T7E1 assay was performed to evaluate indel efficiency at 72h after transduction. We then chose gRNA2, which had the best efficiency, to package in AAV serotype 8 (PackGene Biotech) for administration to the mice.

Case 2

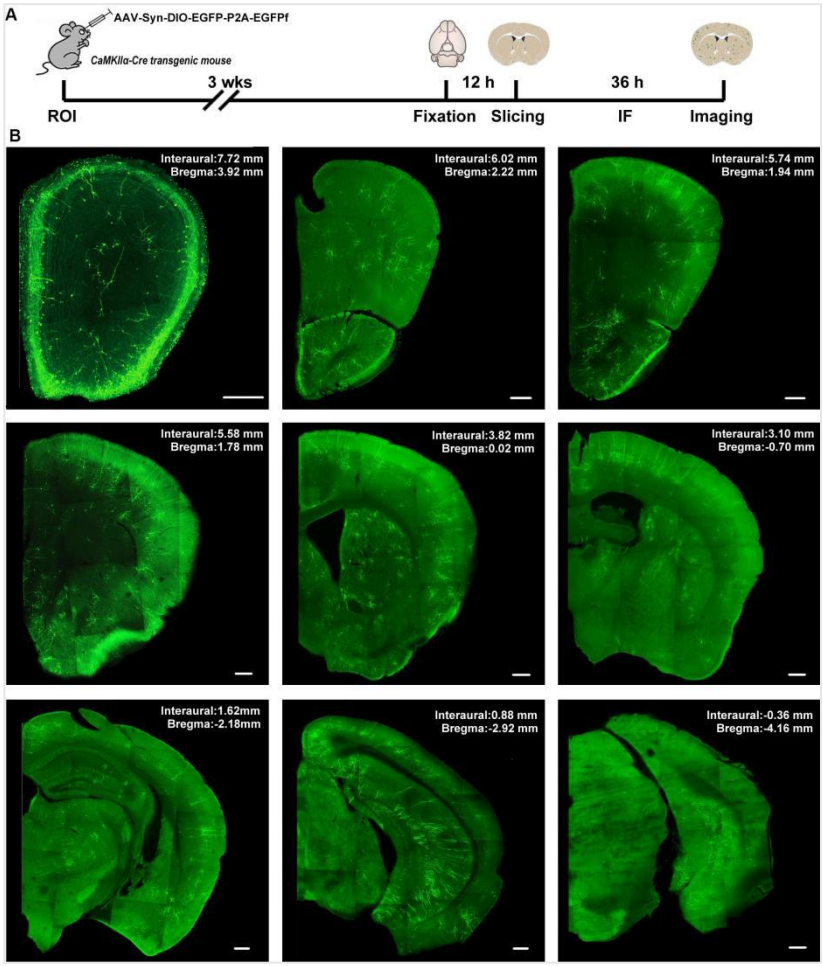
Products: HBV8-B-AAV from PackGene Biotech
Injection Method: Tail Vein Injection
Targeting Site: Liver
Animal Model: C57BL/6
Journal: Nature Neuroscience, 2022 (IF=10.103)
Paper Title: A broad-spectrum nanobody targeting the C-terminus of the hepatitis B surface antigen for chronic hepatitis B infection therapy
DOI: https://doi.org/10.1016/j.antiviral.2022.105265



All mouse experiments were performed according to the guidelines of the Laboratory Animal Management Ethics Committee at Xiamen University. C57BL/6 mice were injected with HBV/adeno-associated virus (AAV) (HBV–B from PackGene Biotech, HBV-D from Five Plus Molecular Medicine Institute, China) via the caudal vein. HCAb treatment was conducted as previously described (Zhou et al., 2020).

Case 3

Products: AAV-PhP.eB from PackGene Biotech
Injection Method: Retro-ocular Injection
Recommended Dose: 1.3E+12GC/ml, 100ul
Targeting Site: Neuron
Animal Model: Shank3B KO mice; Sst-IRES-Cre knock-in mice
Journal: Frontiers in cellular neuroscience, 2020 (IF=6.147)
Paper Title: A whole-brain cell-type-specifific sparse neuron labeling method and its application in a shank3 autistic mouse model
DOI: https://doi.org/10.3389/fncel.2020.00145



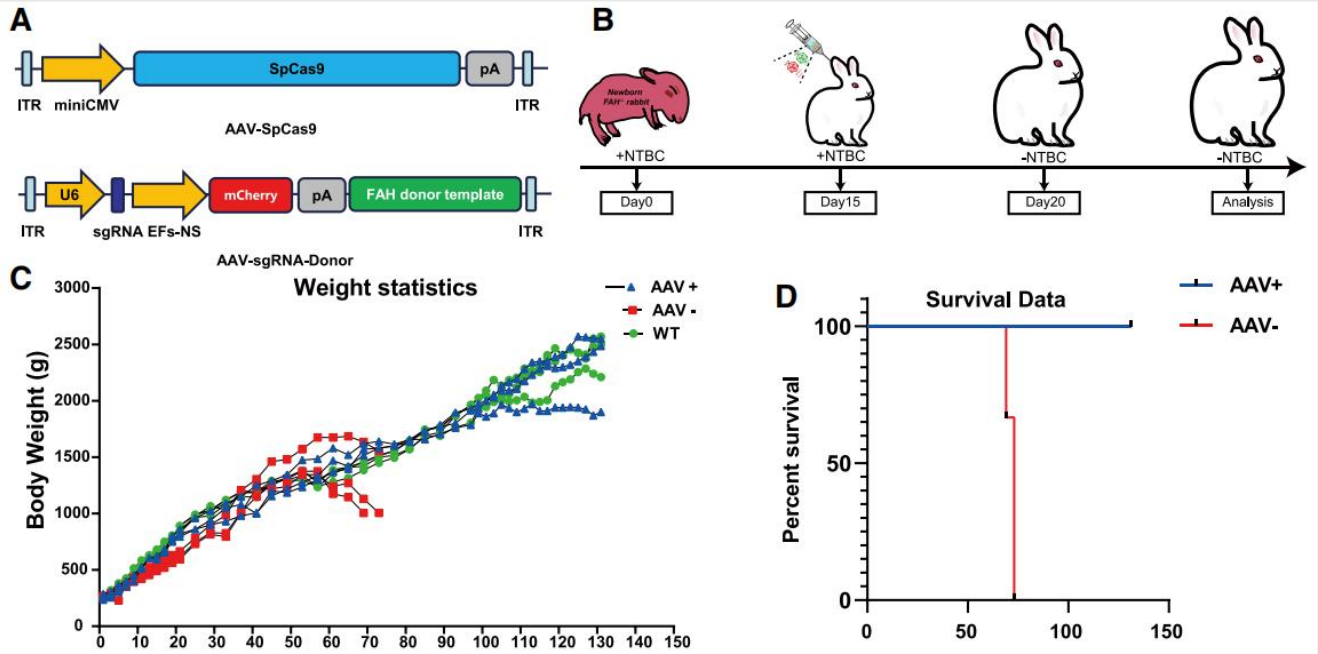
Retro-Ocular Injection

Mice were given retro-ocular injection of AAV as previously described. Briefly, mice were anesthetized with 3% isofluranne. Then, 100μl of AAV php.eb-DIO-EGEP-P2A-EGFPf with a titer of 1.3E+12gc/ml (PackGene Biotech, LLC, China) was injected into the retro-orbital sinus with a 27-G needle and a 1-ml syringe. The mice were then placed in

a warm and moist environment to wait for resuscitation. After resuscitation, the mice were put back in the cage and housed for 3 weeks before the next step.

Case 4

Products: AAV8 from PackGene Biotech
Injection Method: Ear Vein Injection
Recommended Dose: AAV8-SpCas9 3x10^12GC; AAV8-sgRNA4-Donor 6x10^12GC
Targeting Site: Liver
Animal Model: Rabbits
Journal: Molecular Therapy, 2021 (IF=12.910)
Paper Title: CRISPR/Cas9-Mediated Gene Correction in New born Rabbits with Hereditary Tyrosinemia Tpye Ⅰ
DOI: https://doi.org/10.1016/j.ymthe.2020.11.023

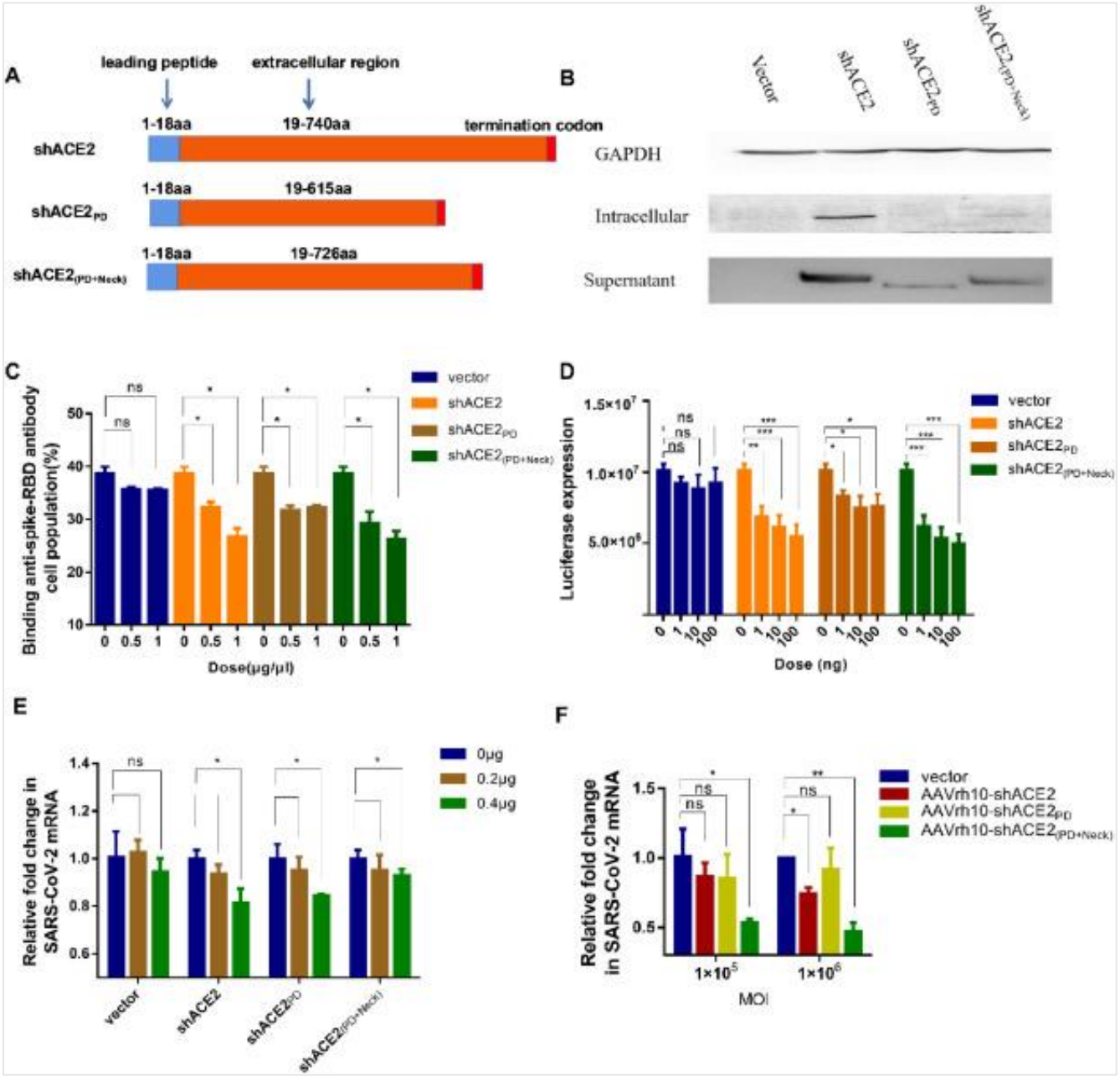


AAV8 Production and Purification

AAV8 was produced and purified by PackGene Biotech. Donor template and optimal sgRNA with U6 promoter were packaged into an AAV8 with mCherry fluorescent reporter, referred to as AAV8- sgRNA4-Donor. SpCas9 with the miniCMV promoter was packaged into the other AAV8, referred to as AAV8- SpCas9. The purified AAV8 was stored in a freezer at 80℃.

Case 5

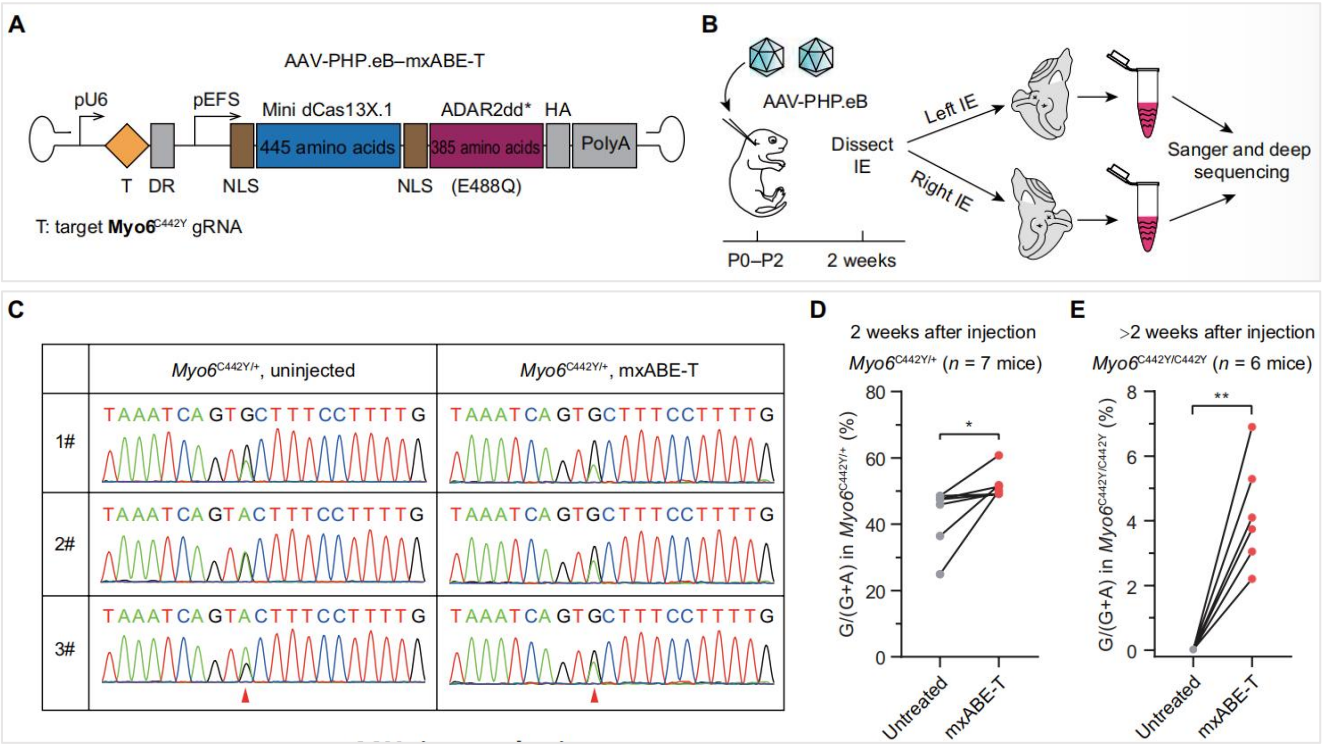
Products: AAVrh10 from PackGene Biotech
Injection Method: Intranasal Administration
Recommended Dose: 2×10^11GC/ml, 40μl
Animal Model: 6-8-week-old female BALB/c mice
Journal: Antiviral Research, 2022 (IF=10.103)
Paper Title: Rational design of AAVrh10-vectored ACE2 functional domain to broadly block the cell entry of SARS-CoV-2 variants
DOI: https://doi.org/10.1016/j.antiviral.2022.105383



AAVrh10 expressing the different ACE2 fragments, including shACE2, shACE2PD, shACE2(PD + Neck), hACE224-83, dACE224-83, were packaged by PackGene Biotech.

Case 6

Products: AAV-PHP.eB from PackGene Biotech
Injection Method: Cochlear Injection
Recommended Dose: 1.43E+10^10GC (2.86×10^13 GC/ml in 500nl/3.99E×10^13 GC/ml in 358.4nl)
Targeting Site: Cochlea
Animal Model: Myo6C442Y/+ and Myo6C442Y/C442Y mice
Journal: Antiviral Research, 2022 (IF=10.103)
Paper Title: Rescue of autosomal dominant hearing loss by in vivo delivery of mini dCas13X-derived RNA base editor
DOI: 10.1126/scitranslmed.abn0449

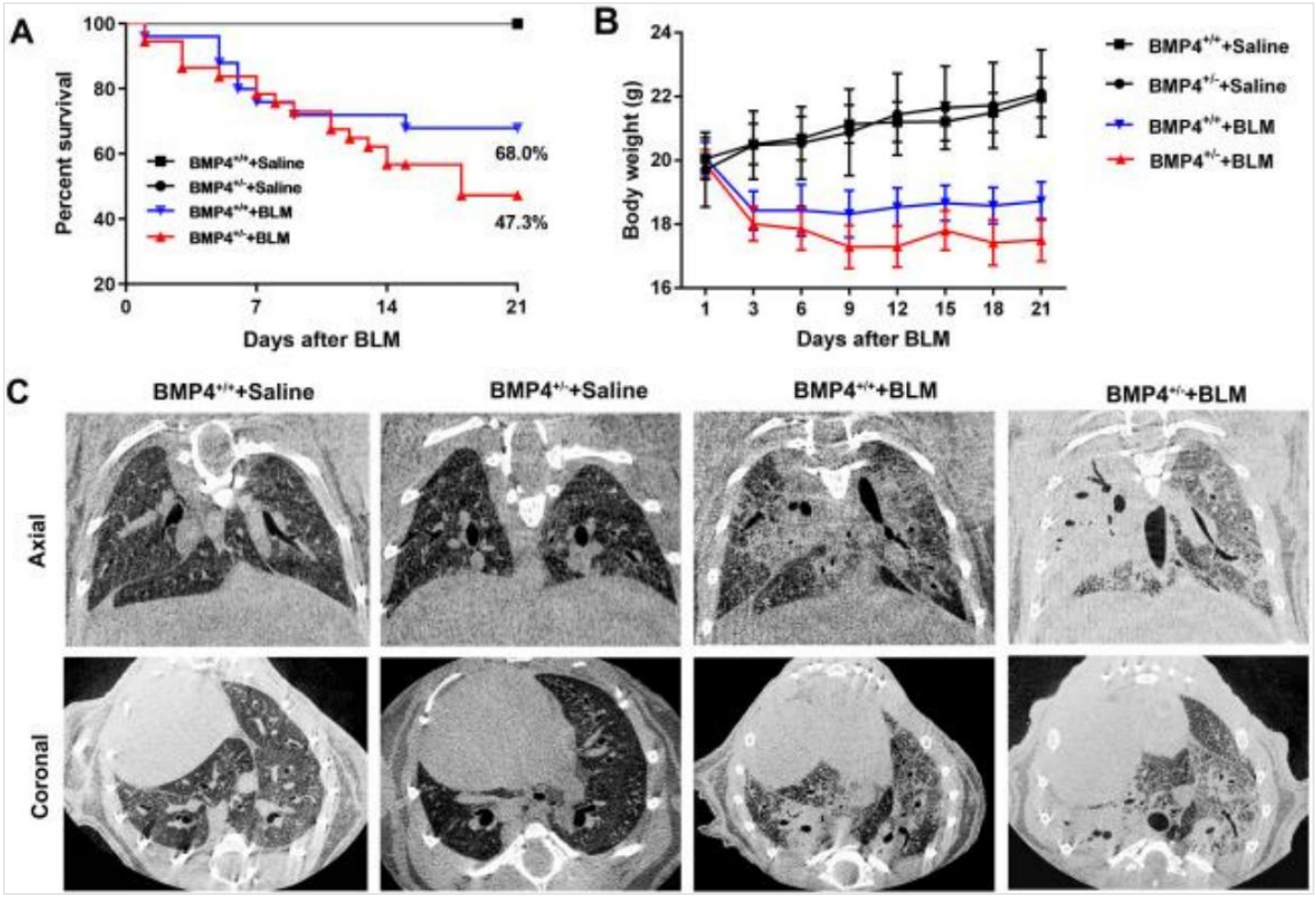


AAV virus production

AAV-PHP.eB serotype (*25*, *35*) was used in this study. The mxABE plasmid with target or nontarget gRNA was sequenced before packaging into AAV-PHP.eB vehicle, and the AAV vectors were packaged by PackGene Biotech. The vector titer was 2.86x10^13 and 3.99x10^13 genome copies/ml as determined by qPCR specific for the in verted terminal repeat of the AAV-PHP.eB–*Myo6* and control virus.

Case 7

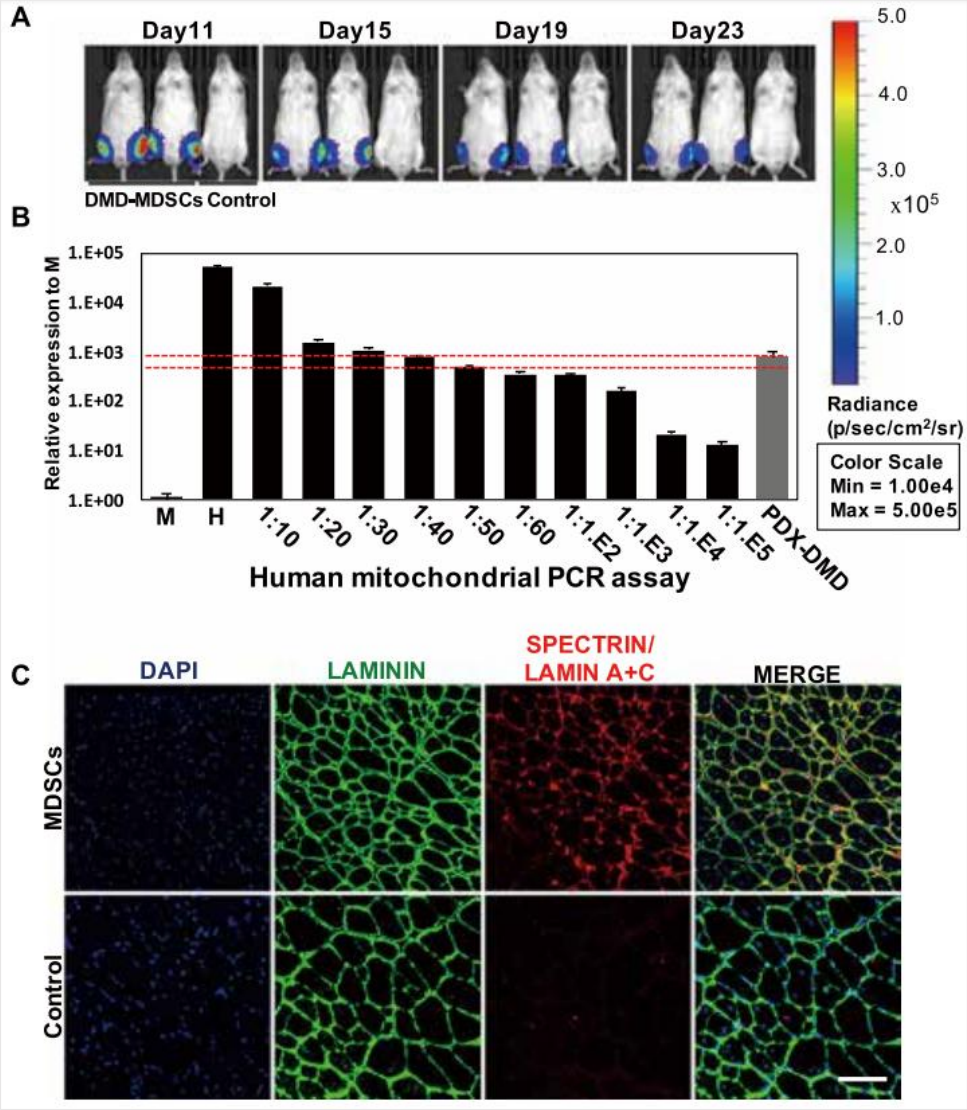
Products: AAV9 from PackGene Biotech
Injection Method: Intratracheal Injection
Recommended Dose: 1×10^10vg/ml
Targeting Site: Lung
Animal Model: C57BL/6 BMP4+/+ and BMP4+/−mice
Journal: European respiratory journal, 2022 (IF=33.795)
Paper Title: Bone morphogenetic protein 4 inhibits pulmonary fibrosis by modulating cellular senescence and mitophagy in lung fibroblasts
DOI: 10.1183/13993003.02307-2021



heterozygous null (BMP4+/−) mice for all the experiments. In addition, separate batches of mice were used for the prevention and therapeutic studies. In the prevention study, mice were administrated with BMP4-expressing AAV9 viral genome particle (1.0×10^10 vg/mL, PackGene Biotech, Guangzhou, China) via intratracheal injections. After 21 days post-viral treatment, these mice were...

Case 8

Products: CRISPR AAV vectors from PackGene Biotech
Injection Method: Intramuscular Injection
Recommended Dose: 25 μL, 1.5E+12 vg/ml
Targeting Site: muscle
Animal Model: DMD mouse model
Journal: Genome Medicine, 2021 (IF=15.266)
Paper Title: In vivo genome editing in mouse restores dystrophin expression in Duchenne muscular dystrophy patient muscle fibers
DOI: 10.1186/s13073-021-00876-0

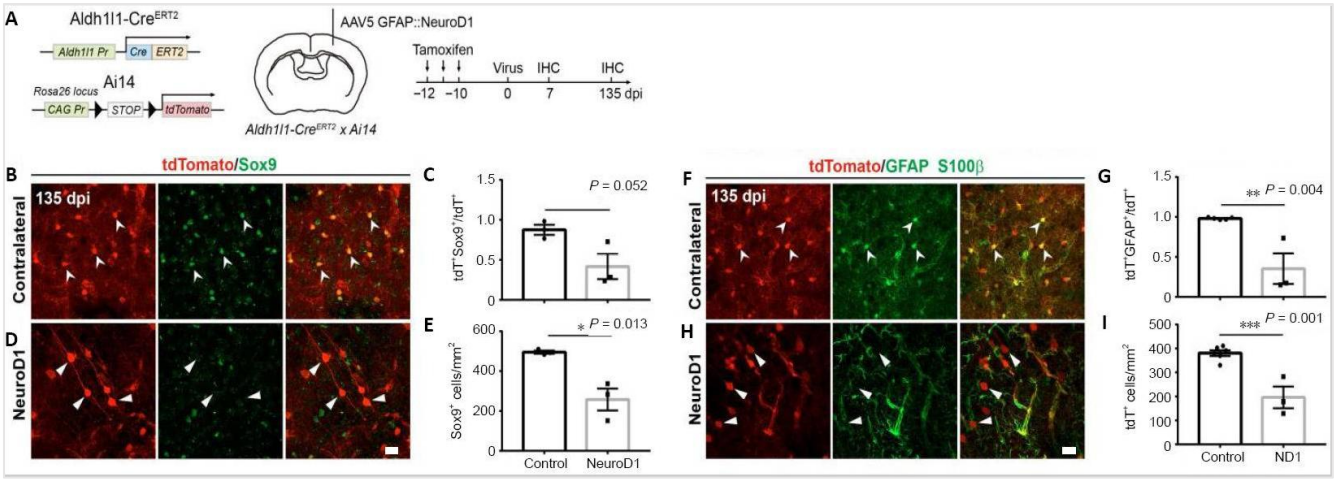


AAV production and injection

CRISPR AAV vectors were generated by PackGene Biotech Co. (Guangzhou, China). For intramuscular injection into NSI mice, the animals were anesthetized by abdominal injection of 5% chloral hydrate, followed by injection of CRISPR AAV vector (25 μL, 1.5E+12 vg) into the TA muscle transplanted with DMD–MDSCs.

Case 9

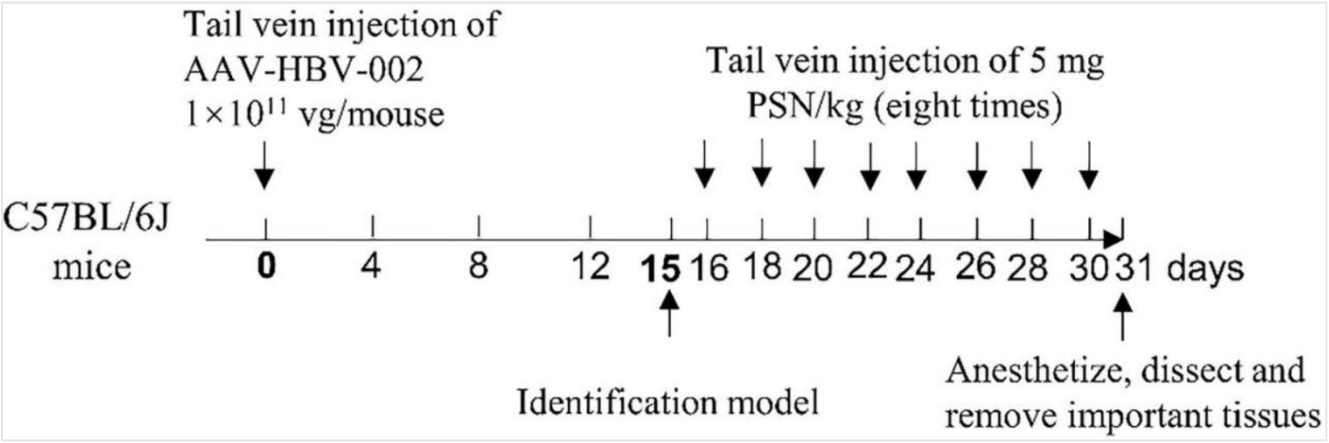
Products: AAV9 and AAV5 from PackGene Biotech
Injection Method: Intramuscular Injection
Recommended Dose: 2×10^13GC/ml, 1μl
Targeting Site: Central Nervous System
Animal Model: Adult C57BL/6J Mice
Journal: Neural Regeneration Research, 2021 (IF=6.058)
Paper Title: Lineage tracing of direct astrocyte-to-neuron conversion in the mouse cortex
DOI: 10.4103/1673-5374.295925



AAV serotype 9 (AAV9) and 5 (AAV5) were produced by PackGene Biotech, LLC, purified through iodixanol gradient ultracentrifuge and subsequent concentration.

Case 10

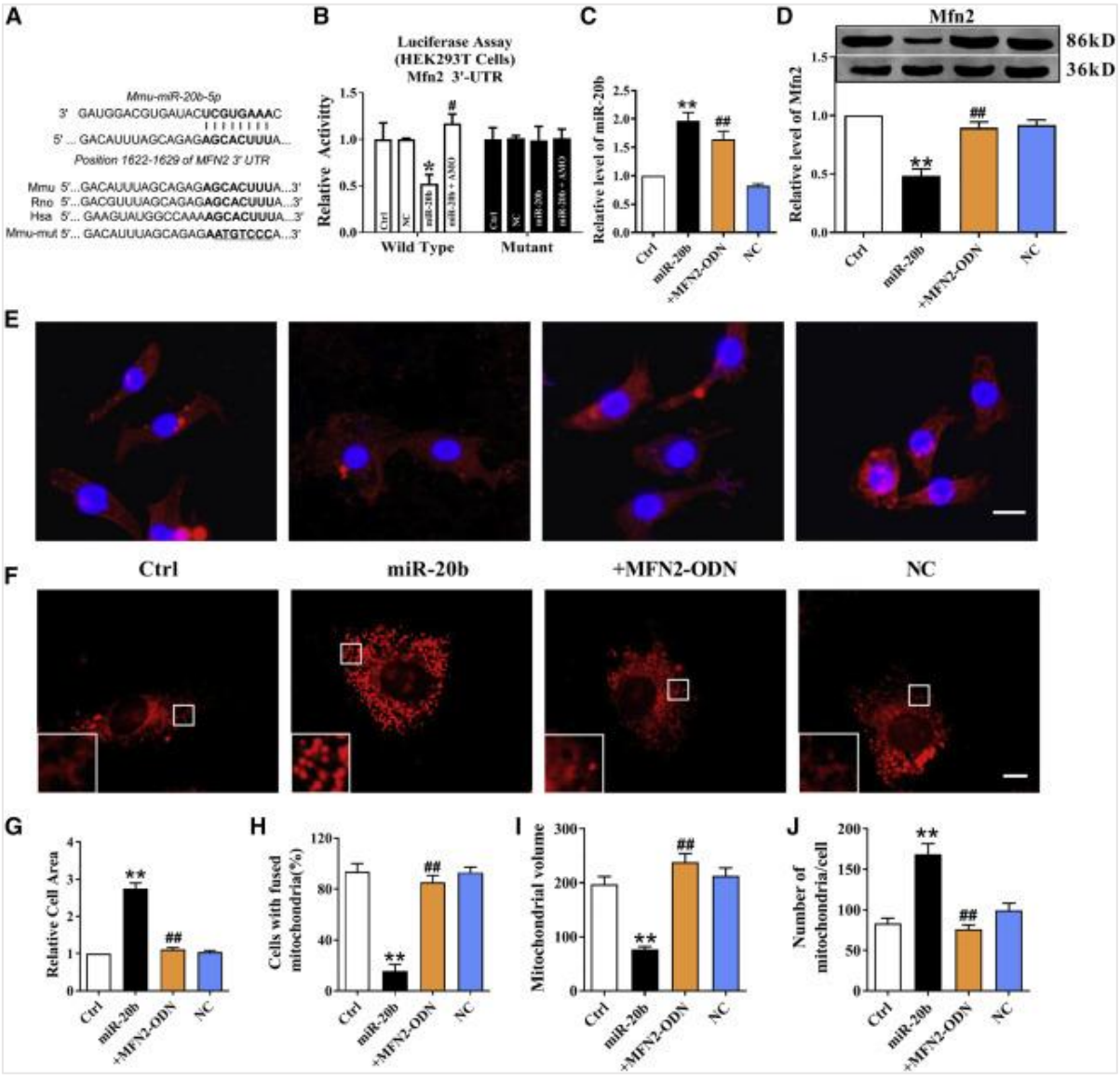
Products: AAV2 from PackGene Biotech
Animal Model: Hepatitis B Model Mice
Journal: Frontiers in Immunology, 2022 (IF=8.786)
Paper Title: PreS/2-21-Guided siRNA Nanoparticles Target to Inhibit Hepatitis B Virus Infection and Replication
DOI: 10.3389/fimmu.2022.856463



The recombinant adeno-associated virus AAV-HBV-002 applied to construct the hepatitis B model was purchased from PackGene Biotech, Co., Ltd. AAV-HBV-002 containing 1.3× HBV genome, is characterized by the production of HBV DNA, HBeAg and HBsAg, and the genotype is C2 and serotype is adr. Sixteen mice

Case 11

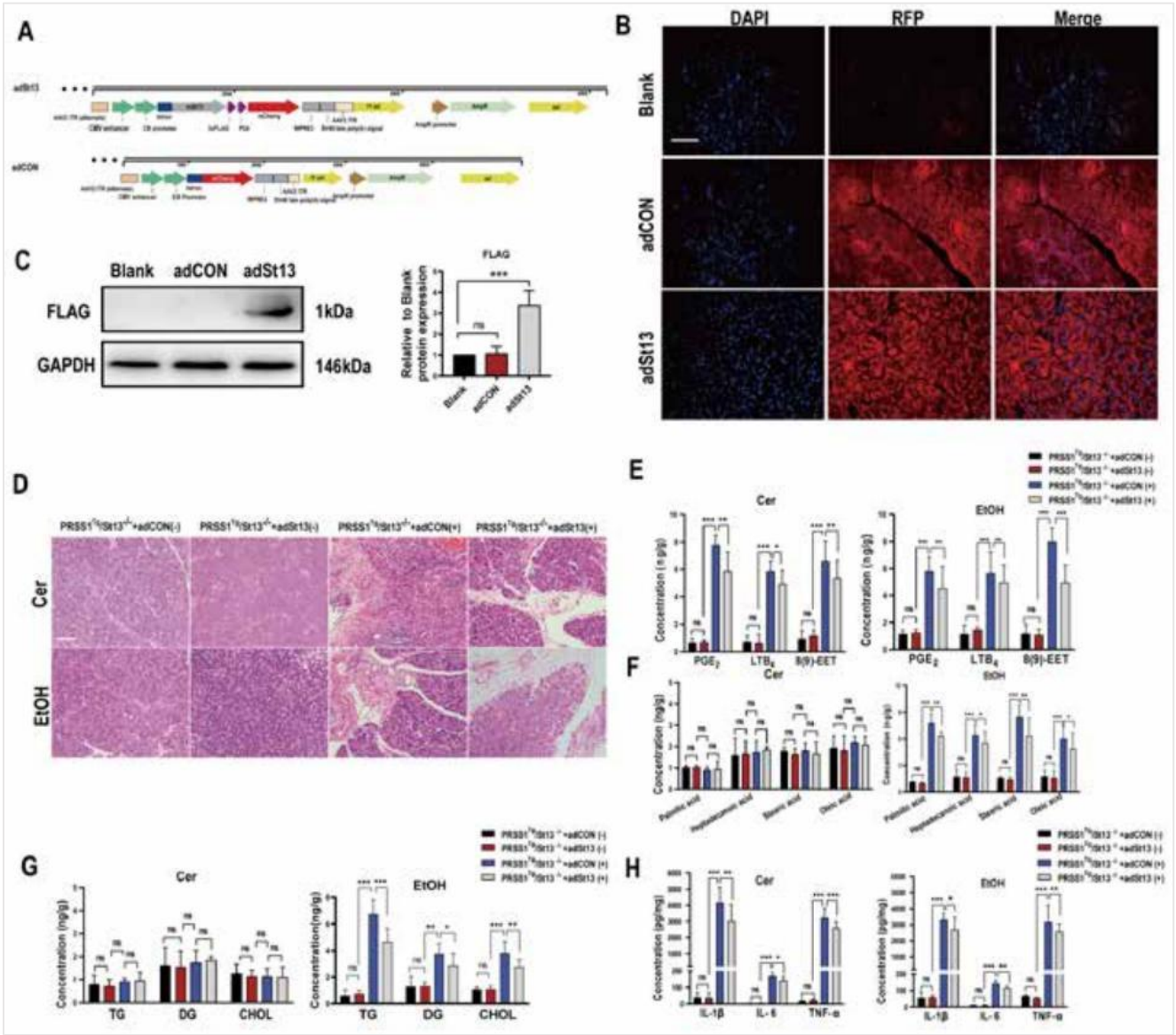
Products: AAV9 from PackGene Biotech
Injection Method: Tail Vein Injection
Recommended Dose: 1×10^11vg/mouse
Targeting Site: Heart
Animal Model: Adult Male Kunming Mice (weight 22–25g)
Journal: Molecular Therapy Nucleic Acids, 2020 (IF=10.183)
Paper Title: MicroRNA-20b Promotes Cardiac Hypertrophy by the Inhibition of Mitofusin 2-Mediated Inter-organelle Ca2+ Cross-Talk
DOI: https://doi.org/10.1016/j.omtn.2020.01.017



Synthesis and Administration of rAAV9-anti-miR-20brAAV was used as carrier, among which rAAV9 is the most efficient vector for myocardial transduction. For this regard, rAAV9-antimiR-20b or rAAV9-NC was produced (PackGene Biotech, Guangzhou, China) and the sequences were delivered into mouse, respectively, through tail vein injection at 1- 10^11vg (viral genomes) per animal at 5 weeks before TAC. Animals were divided into four

Case 12

Products: All AAV from PackGene Biotech
Targeting Site: Pancreas
Animal Model: CP Model Mice
Journal: Journal of Translational Medicine, 2022 (IF=8.440)
Paper Title: St13 protects against disordered acinar cell arachidonic acid pathway in chronic pancreatitis
DOI: 10.1186/s12967-022-03413-8



Blank adenovirus was used as negative control. All adeno-associated virus (PackGene Biotech, China) used are listed in Additional file 4: Fig. S4. Please see the Supplementary materials and methods for more details.