Advances in viral vector delivery

Journal Club

28.01.2020

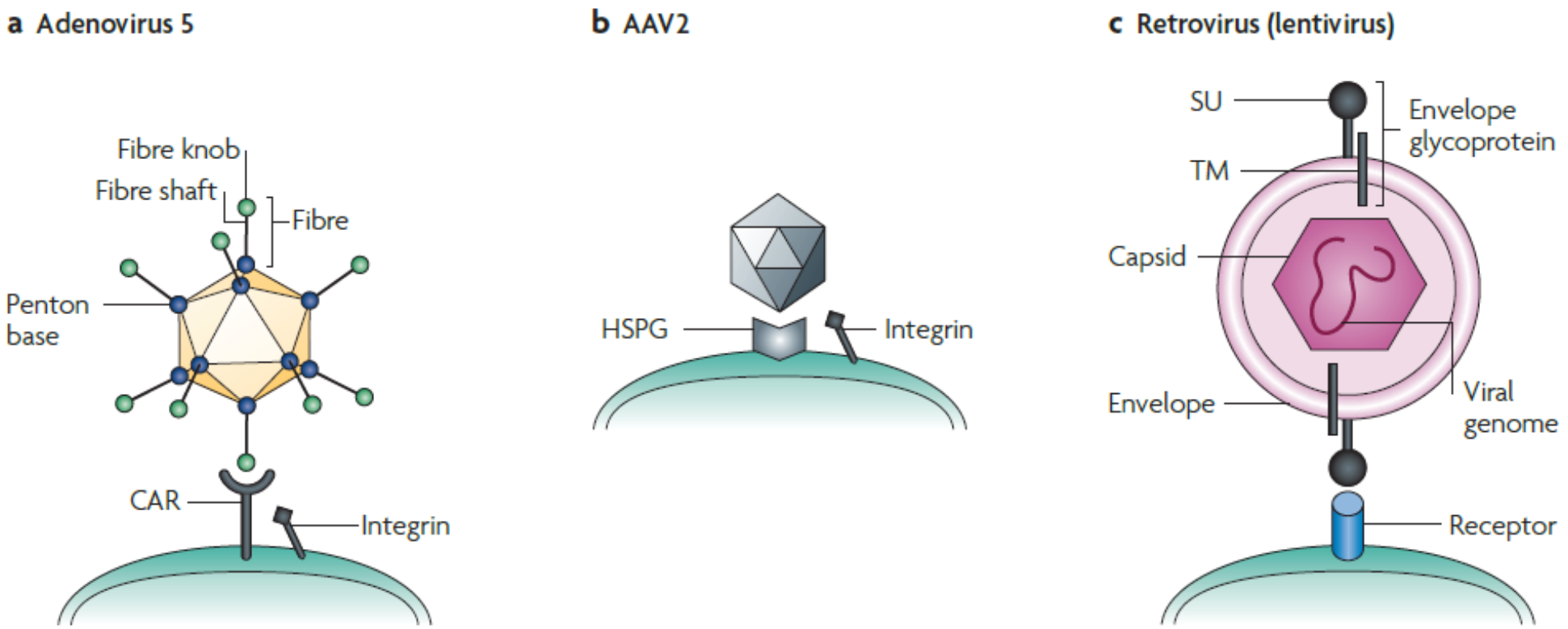
Journal Club - Outline

- Overview: main viral delivery systems and viral vector targeting

- Engineering of AAVs with novel tropism
- Novel hybrid vector for delivery of large cargoes

Most widely used viral delivery systems

Viruses represent powerful tools to deliver genes into host cells. Viruses with different capacities and characteristics have been exploited.



Waehler, R., Russell, S. & Curiel, D. Engineering targeted viral vectors for gene therapy.

Most widely used viral delivery systems

Feature	Adenoviral vector	Helper-dependent adenoviral vector	AAV vector	Retroviral vector	Lentiviral vector
Particle size (nm)	70–100	70–100	20–25	100	100
Cloning capacity (kb)	8–10	~30	4.9 (10 after heterodimerization of two AAV virions)	8	9
Chromosomal integration	No	No	No (yes if *rep* gene is included)	Yes	Yes
Vector yield (transducing units/ml)	High (10^{12})	High (10^{12})	High (10^{12})	Moderate (10^{10})	Moderate (10^{10})
Entry mechanism	Receptor (CAR)-mediated endocytosis, endosomal escape and microtubule transport to the nucleus		Receptor-mediated endocytosis, endosomal escape and transport to the nucleus	Receptor binding, conformational change of Env, membrane fusion, internalization, uncoating, nuclear entry of reverse-transcribed DNA	

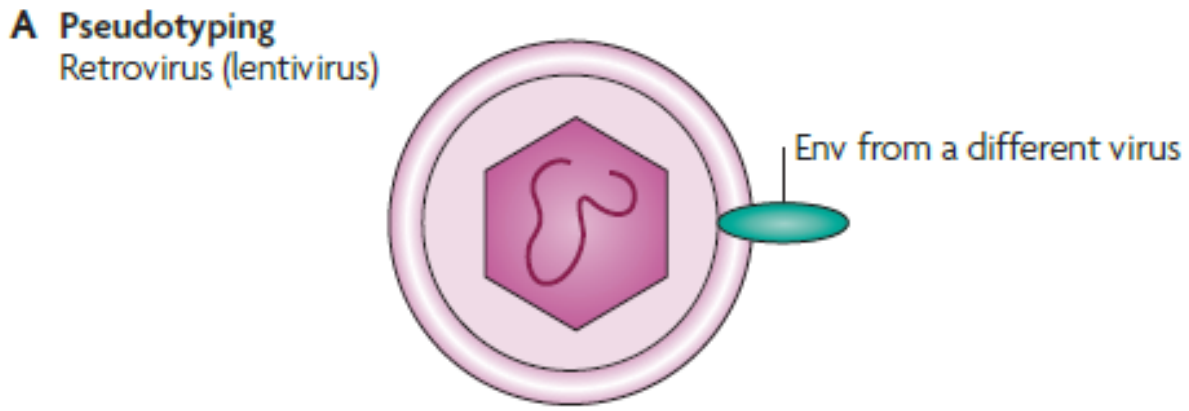
Viral tropism constrains applicablility

One major challenge in the use of viruses for gene delivery is that the viral vector targets the desired cells.

Ways to circumvent the problem:

- Vector targeting by pseudotyping
- Vector targeting using adaptors
- Genetic incorporation of targeting ligands

Vector targeting by pseudotyping



Pseudotyping: Changing the tropism of a virus by replacing the viral attachment protein with that of a related virus. Can be achieved by co-expressing the necessary attachment protein.

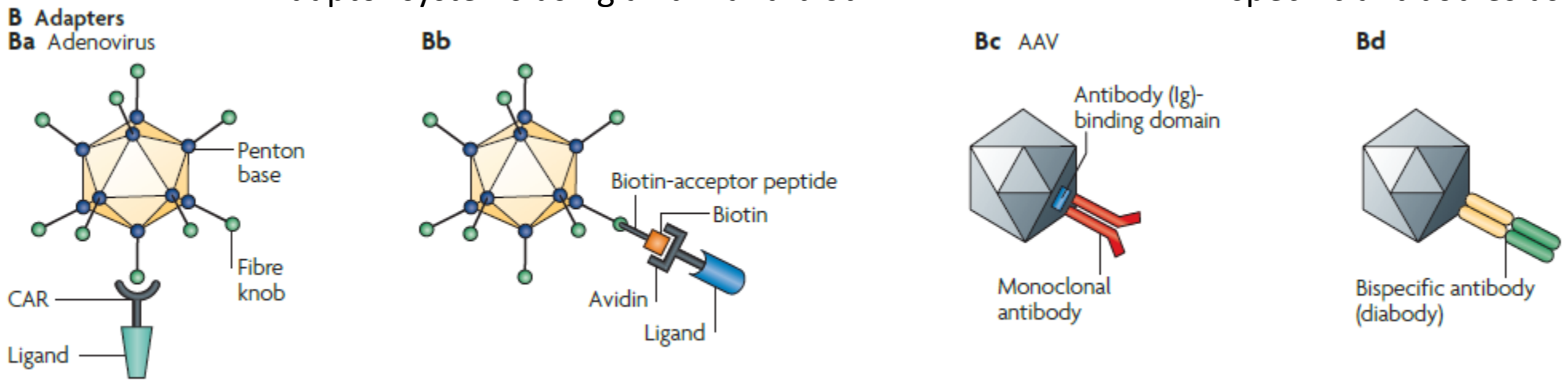
Vector targeting using adaptors

Receptor–ligand complexes

Monoclonal antibodies as adaptors

Adaptor systems using avidin and biotin

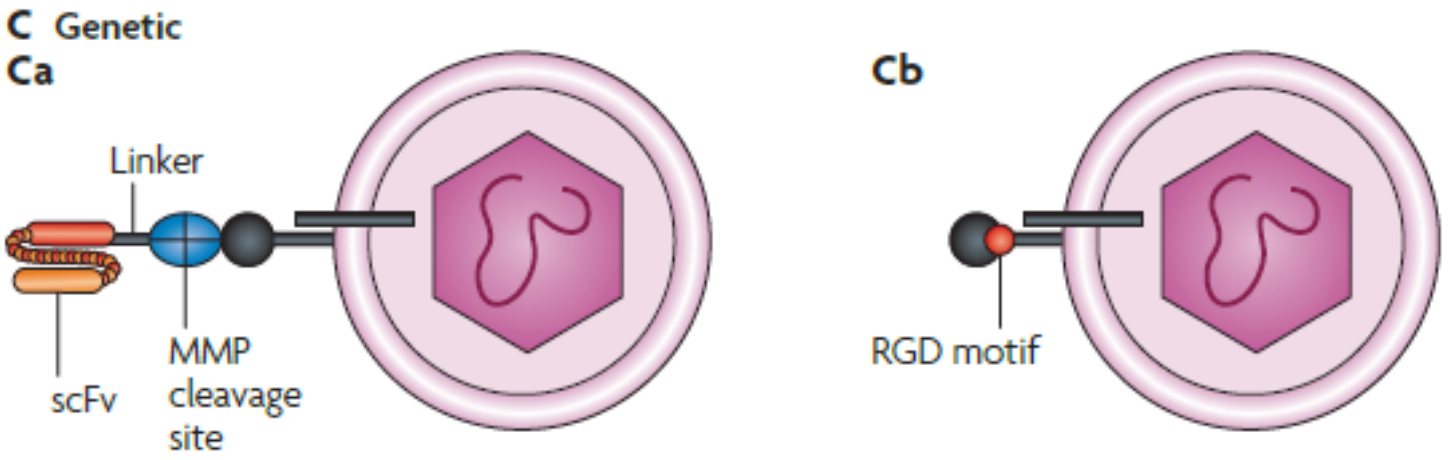
Bispecific antibodies as adaptors



Two-component systems

Viral vector targeting is mediated by a second component

Genetic incorporation of targeting ligands



Via genetical engineering a polypeptide is incorporated into the vector to facilitate targeted transduction

Most widely used viral delivery systems

Limitations:

- Small capacity for cargo.
  - Can deliver genes below a certain size
  - Cannot deliver proteins
- (limited) Tropism
- Preexisting immunity
- safety concerns

Following papers show two approaches how to modulate viral delivery for specific needs

TECHNICAL REPORTS

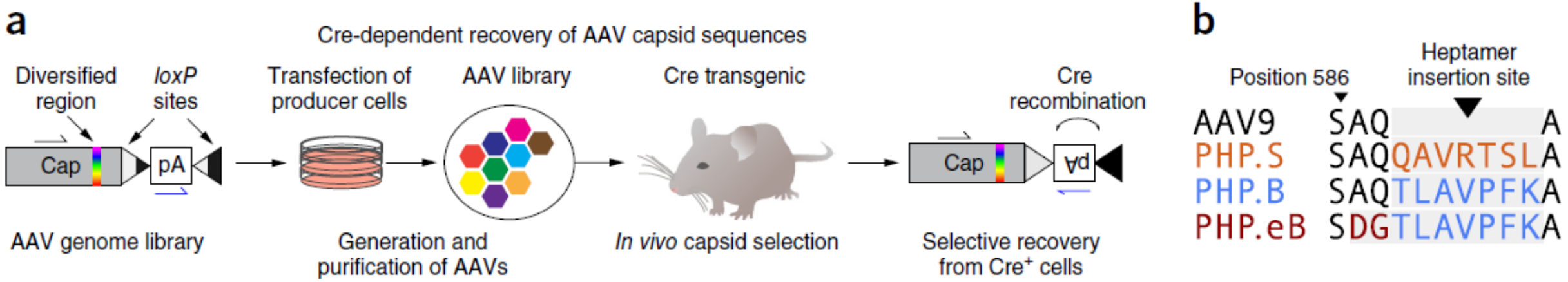
nature neuroscience

Engineered AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems

Ken Y Chan, Min J Jang, Bryan B Yoo, Alon Greenbaum, Namita Ravi, Wei-Li Wu, Luis Sánchez-Guardado, Carlos Lois, Sarkis K Mazmanian, Benjamin E Deverman & Viviana Gradinaru

Description of the development of modulated AAVs to target the CNS or peripheral neurons upon **systemic** delivery

Selection of AAVs with different tropism

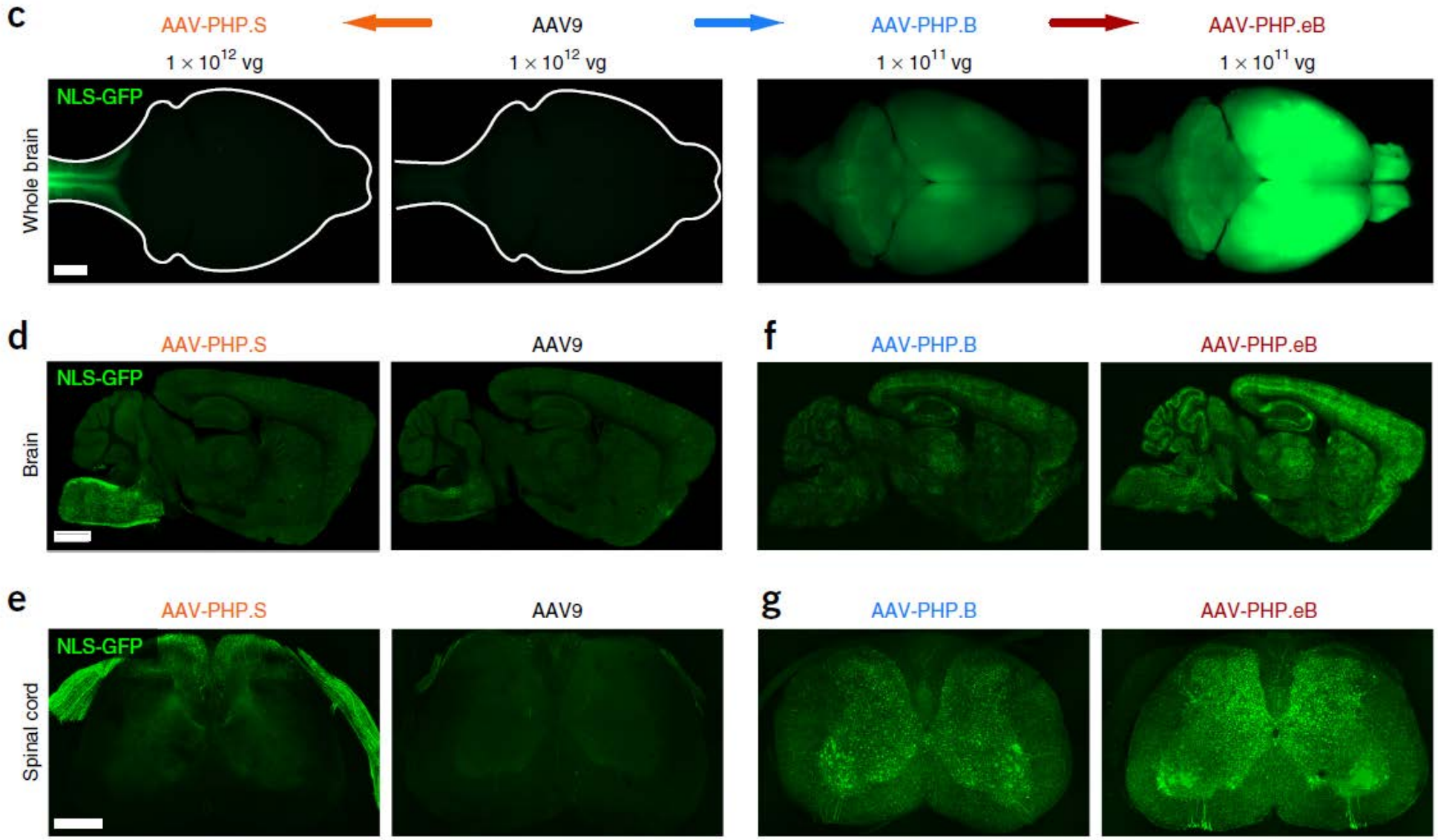


Engineering/Selection pipeline for enhanced neuron/astrocyte and peripheral neuron specific transduction

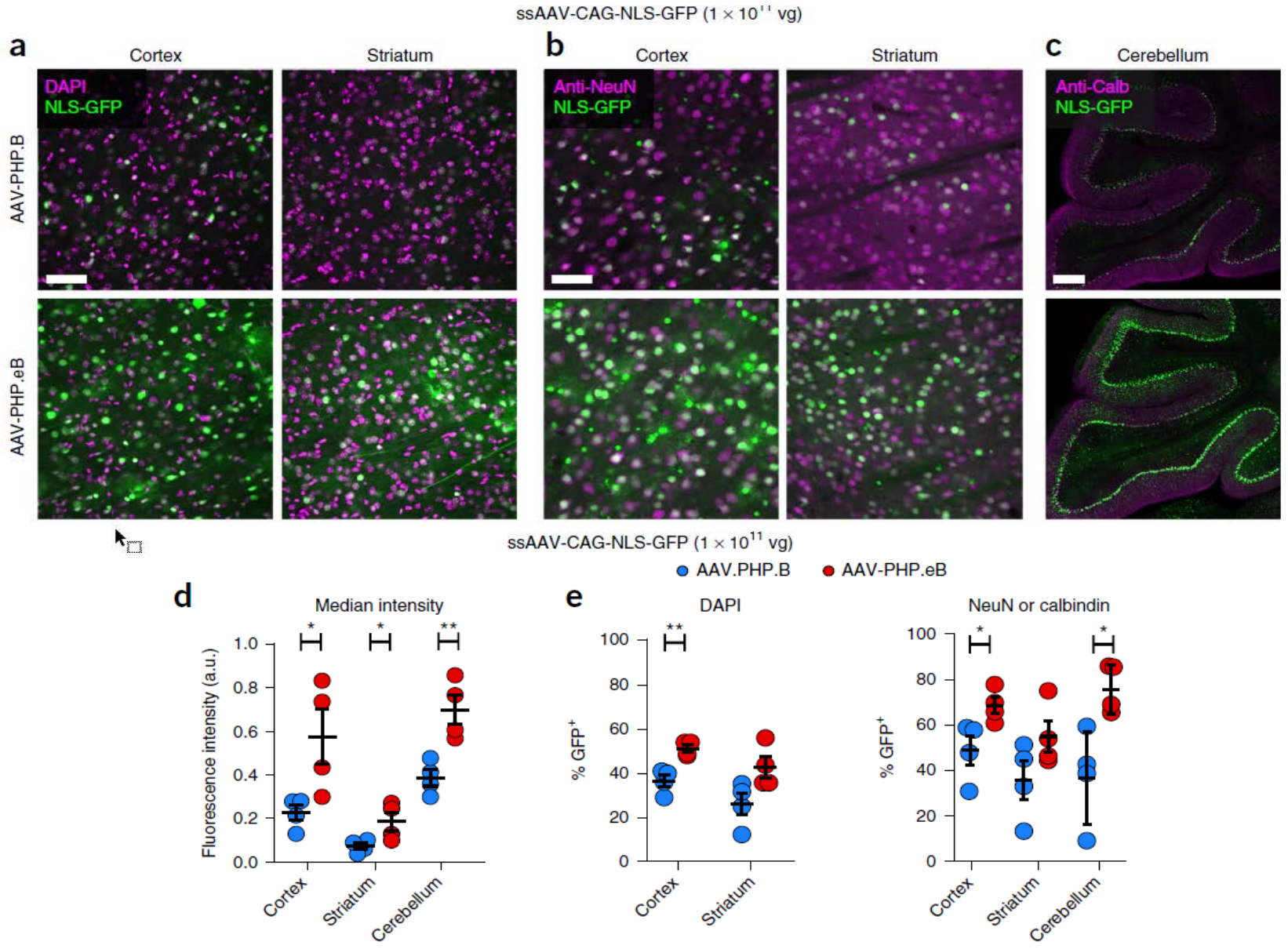
Original AAV9 has been shown to be able to cross the BBB and first round of selection resulted in AAV-PHP.B. (Deverman BE et al., Nat. Biotech., 2016).

Here, they further mutated the responsible hepamter sequence to screen for enhanced tropism => **AAV-PHP.eB**
In addition, they screened AAV9 for in GFAP-Cre mice and discovered a AAV specifically targeting sensory neurons => **AAV-PHP.S**

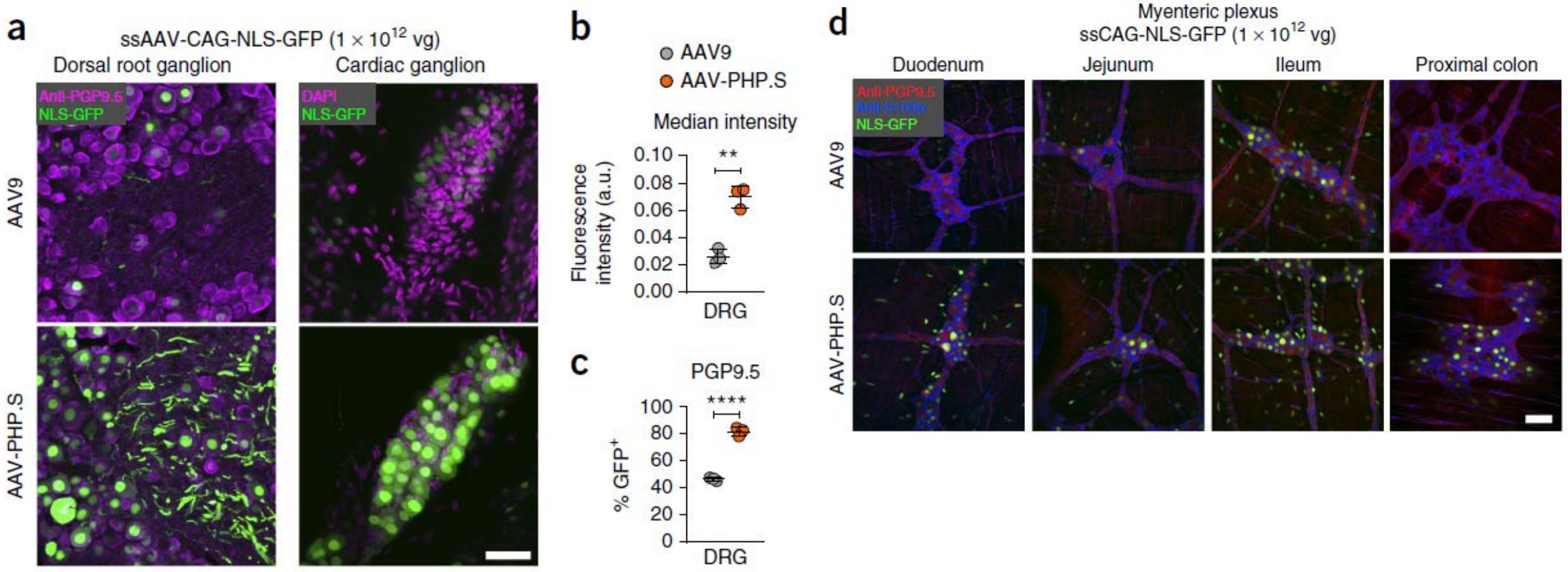
Expression analysis



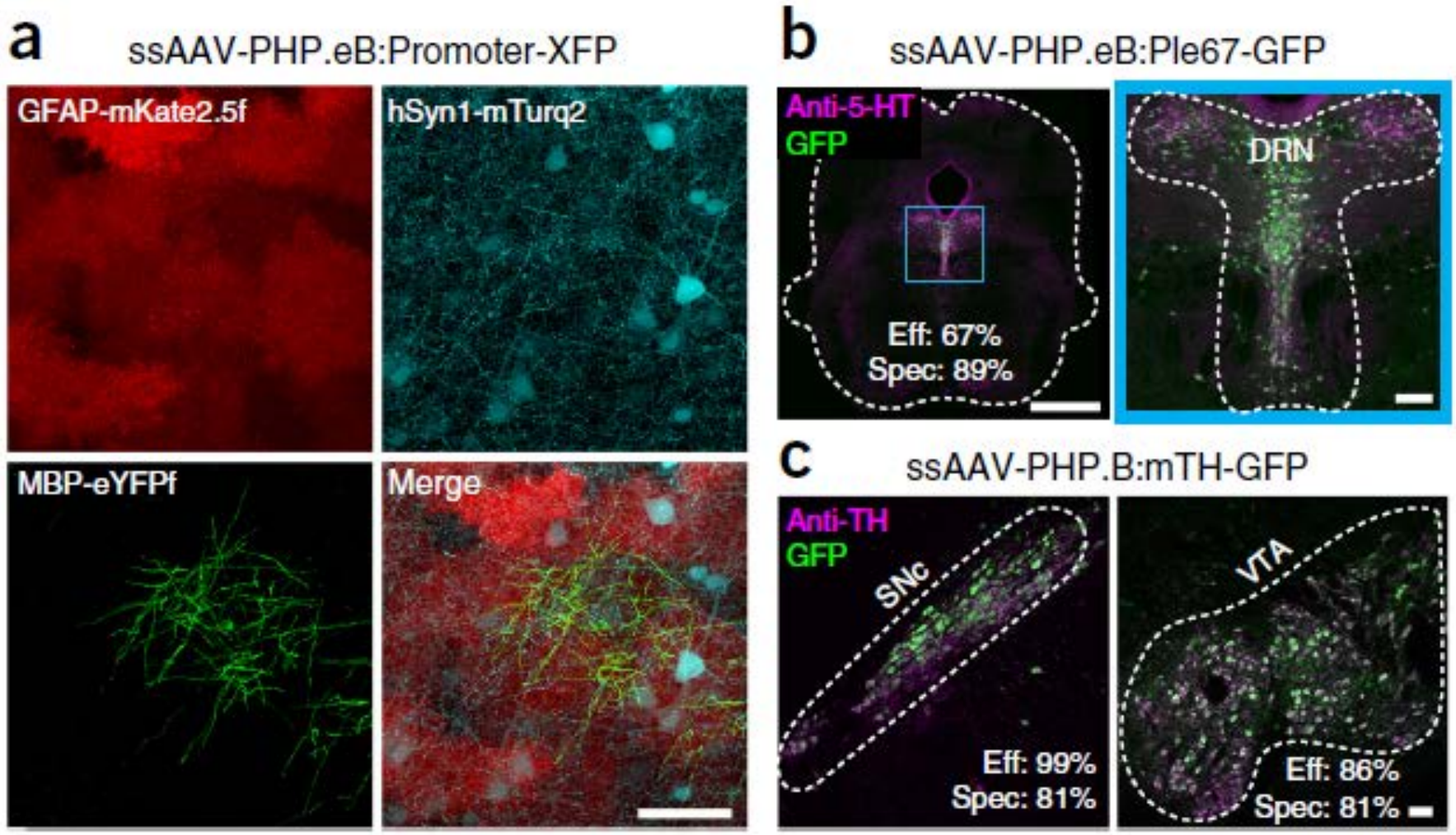
Comparison to previous CNS AAV



Novel PNS neuron specific AAV



Cell type specific AAV delivered gene expression



Conclusions

- Developed and characterized two new capsids, AAV-PHP.eB and AAV-PHP.S, that enable efficient and noninvasive gene delivery throughout the CNS or PNS
- Expression can be restricted to cell types via specifc promoters

- Direct modulation of viruses remains a feasible way to expand their capabilities and exploit the power of viral gene delivery

- However, for parental AAV-PHP-B there is now a active debate ongoing, if the virus only targets the CNS in C57BL/6J mice…

SCIENCE ADVANCES | RESEARCH ARTICLE

VIROLOGY

A prokaryotic-eukaryotic hybrid viral vector for delivery of large cargos of genes and proteins into human cells

Jingen Zhu[1], Pan Tao[1], Marthandan Mahalingam[1], Jian Sha[2], Paul Kilgore[2], Ashok K. Chopra[2], Venigalla Rao[1]*

Description of the development of a prokaryotic-eukaryotic hybrid viral vector to combine AAV tropism with large cargo delivery of bacteriophages

Short intro to T4 - bacteriophage

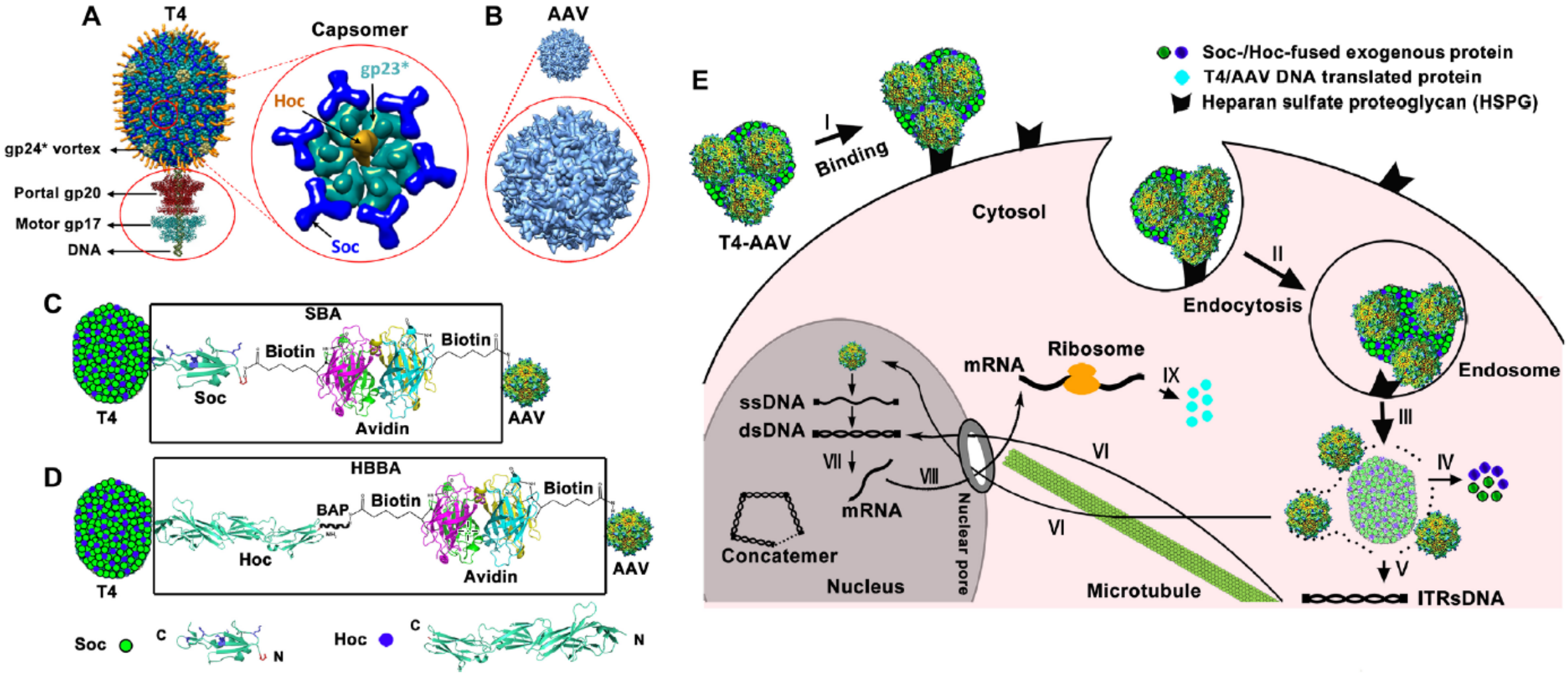
Selected phage	Morphology	Type of genetic material	Size	Host	M.W. of intact phage
T4		ds DNA	200 nm	*E. coli*	110 kD

- Species of bacteriophages that infects *E. coli*
- Empty protein shell (capsid) can be produced individually and packaged with DNA
- Capsid proteins can be modified to display proteins
- The DNA packaging mechanism of the T4 phage is very rapid and powerful (up to ~2000 bp/s)
- Can be packaged with ~170 kb of foreign genes
- Can display (more than 1000) molecules on the capsid surface.

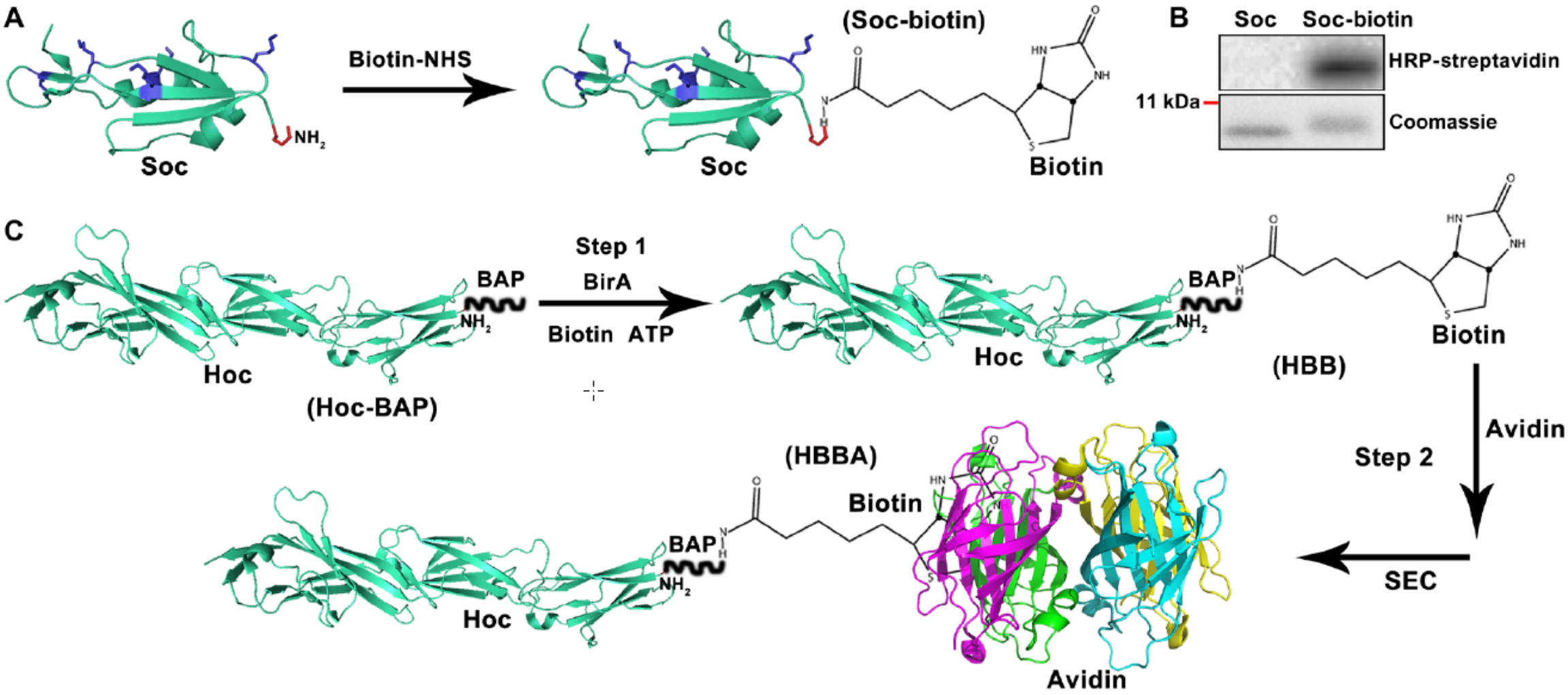
- T4 head has no tropism to human cells, has no known toxicity or pathogenicity, exhibits no preexisting immunity, and can be inexpensively produced on a large scale
- However, phages are poor delivery vehicles because they lack natural mechanisms to enter mammalian cells or reach appropriate intracellular compartments following entry

Source: Karimi et al., 2016, Advanced Drug Delivery Reviews

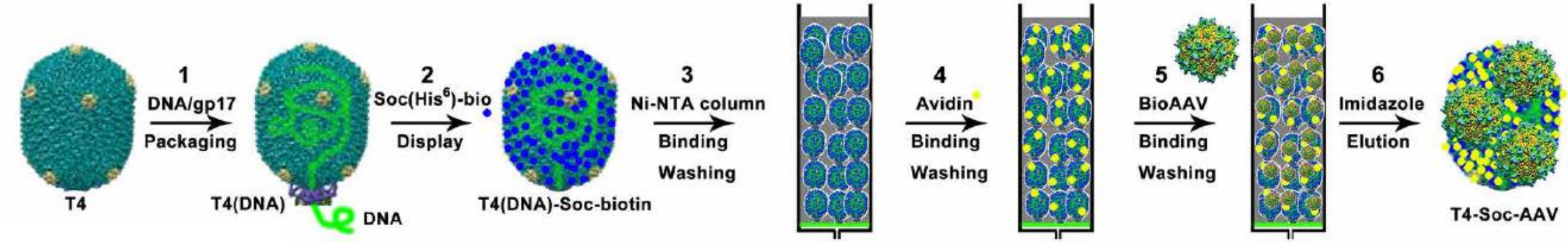
Hybrid vector principal

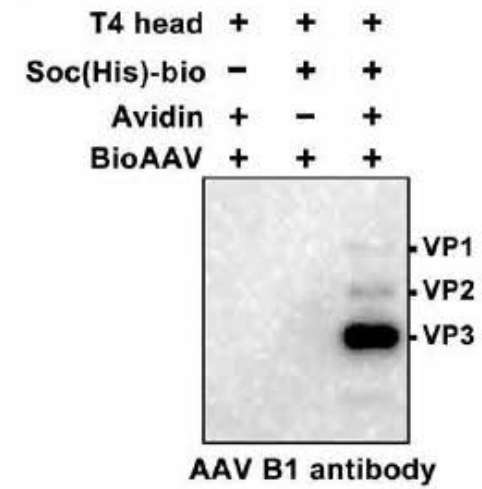


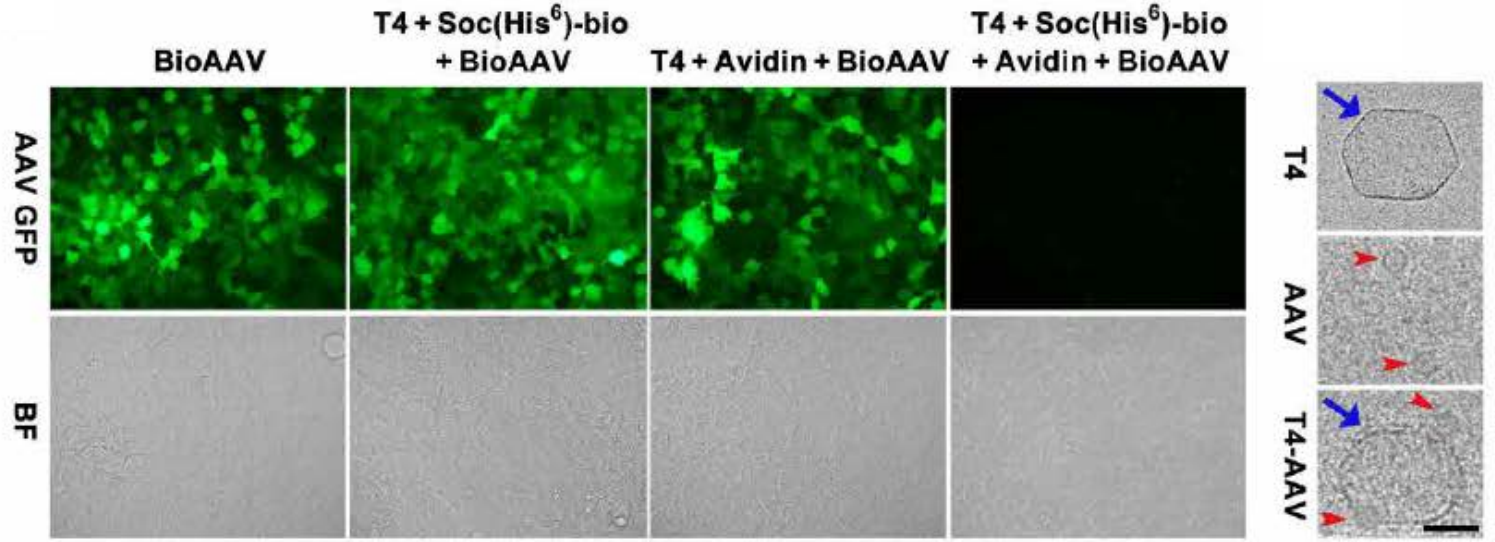
Biotinylation of Soc and Hoc



Coupling to biotinylated-AAV via avidin

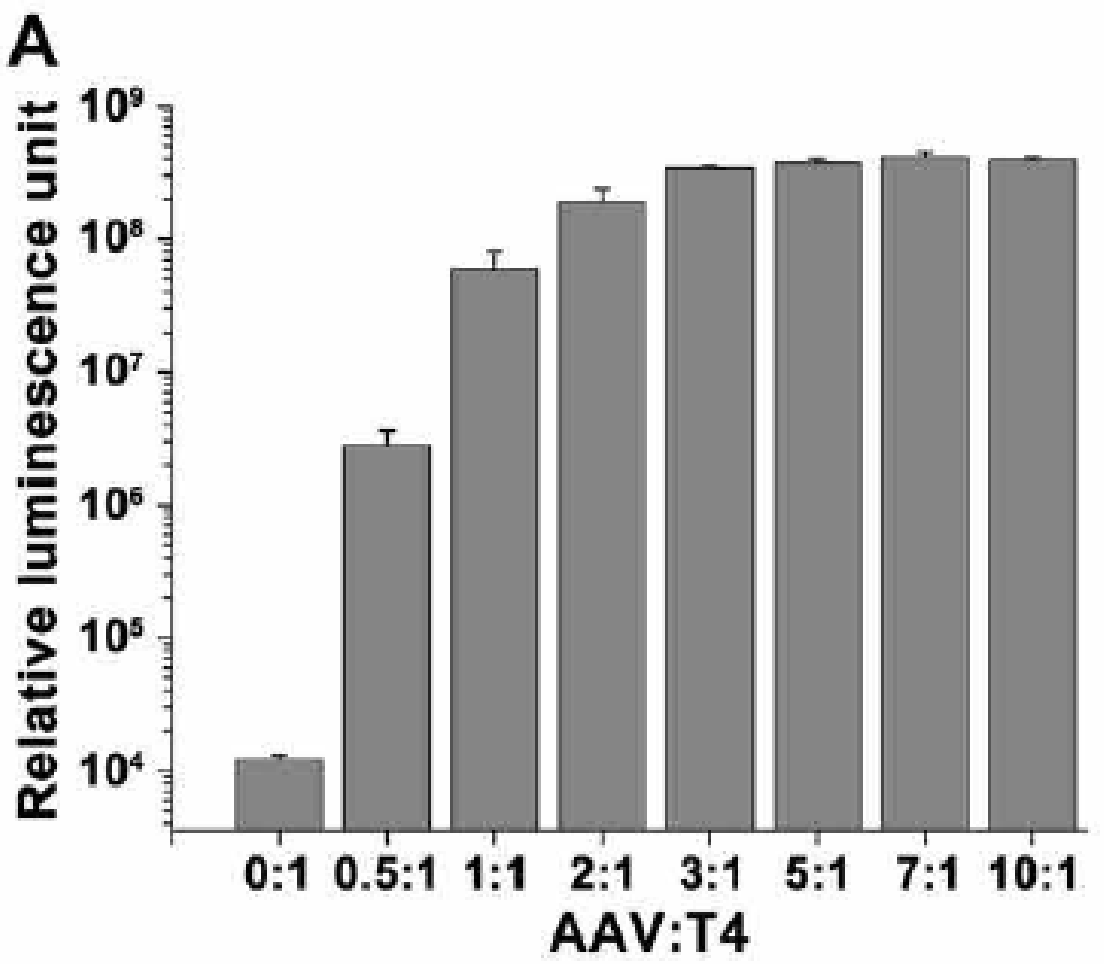






T4-AAV nanoparticles efficiently delivered genes and proteins into mammalian (human) cells

Delivery of around 10 molecules of luciferase plasmid DNA in one T4 head using T4-Soc-avidin (TSA) coupled to AAV

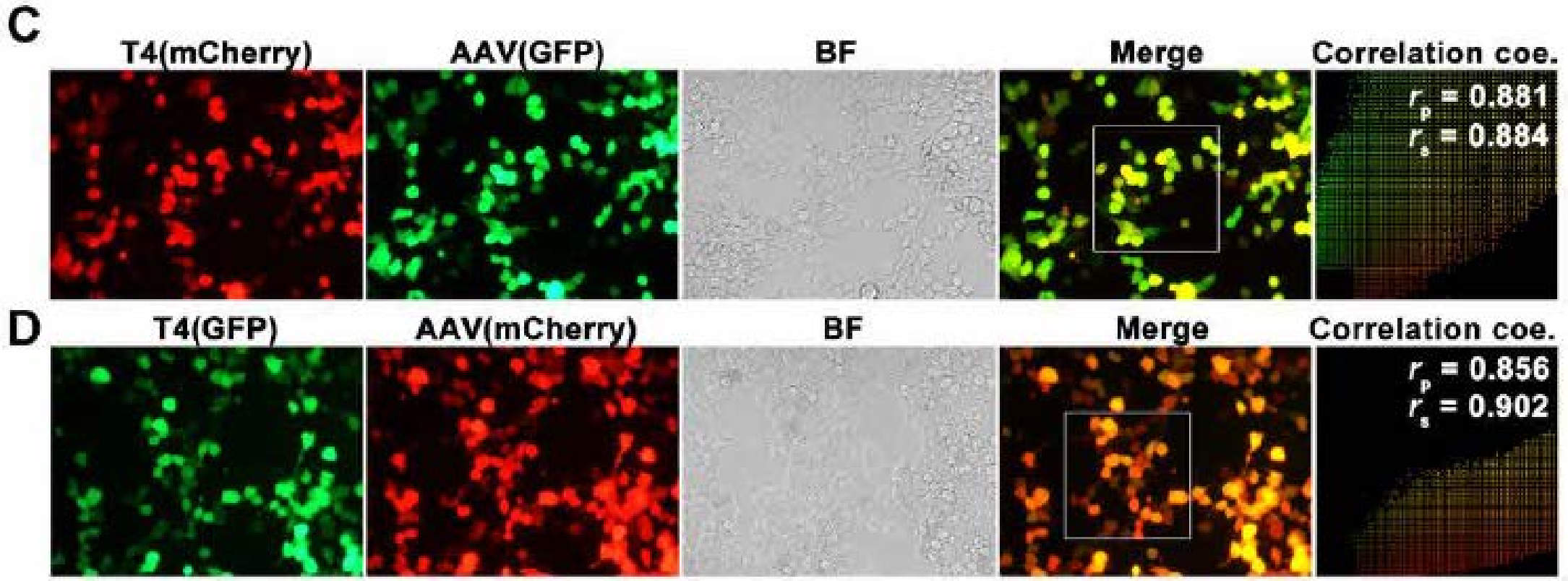


Application of T4 itself shows a very weak Transduction efficeny,

Gene delivery through both T4 and AAV

Both T4 and AAV can be simultaneously used to deliver genes

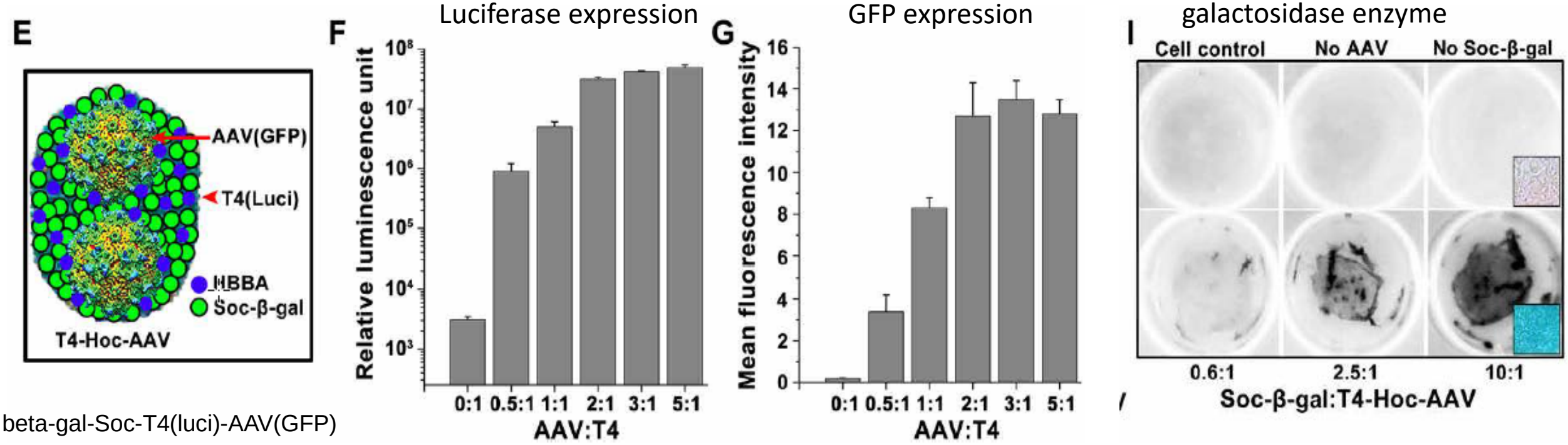
T4(mCherry)-AAV(GFP) nanoparticles (and vice versa) were added to HEK293 cells



Simultaneous delivery of genes and proteins

- 116kDa beta-galactosidase was fused to Soc and displayed on the T4 head (~250 molecules)
- Additionally, ~9 plasmid DNA molecules for luciferase (6.2kbp) were packaged into the head
- At the same time, AAV was packaged with GFP and attached to the T4 head via Hoc bridges
- Transduction into HEK293 cells

successful formation of the functional tetrameric beta-galactosidase enzyme

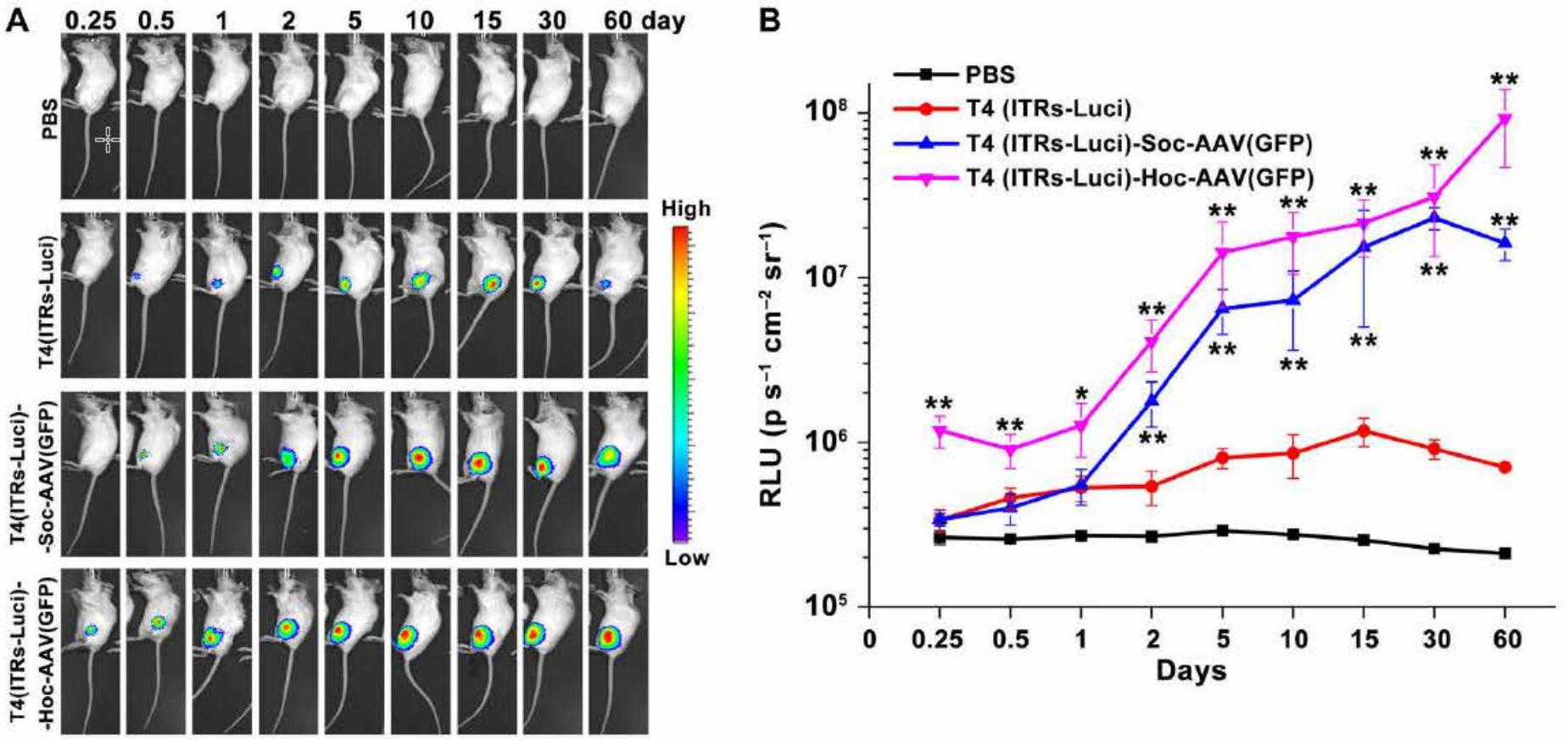


beta-gal-Soc-T4(luci)-AAV(GFP)

the entire cargo of luciferase plasmids, GFP DNA, and beta-galactosidase proteins was efficiently delivered into the cells.

Efficient in vivo gene delivery by T4-AAV nanoparticles

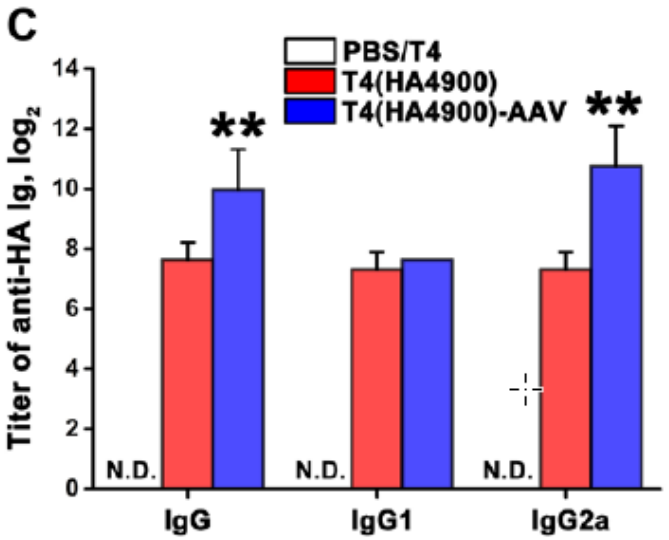
T4-Soc-AAVs and T4-Hoc-AAVs loaded with Luciferase plasmid DNA (in the T4 head) were injected into muscles and showed greater efficency and longer lasting signal than T4 alone

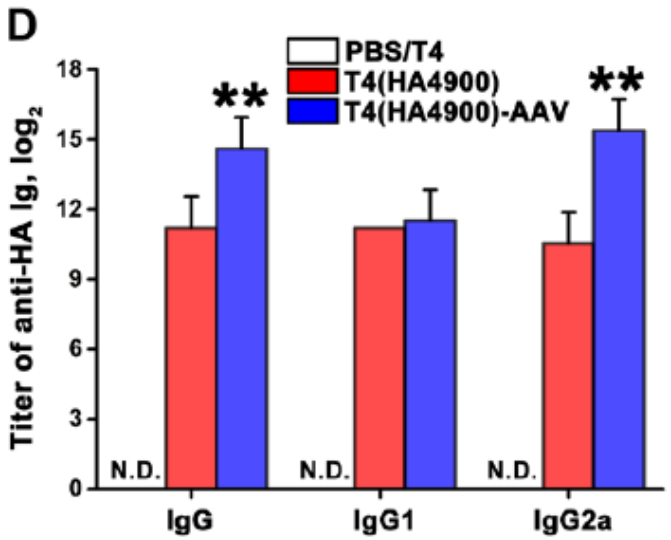


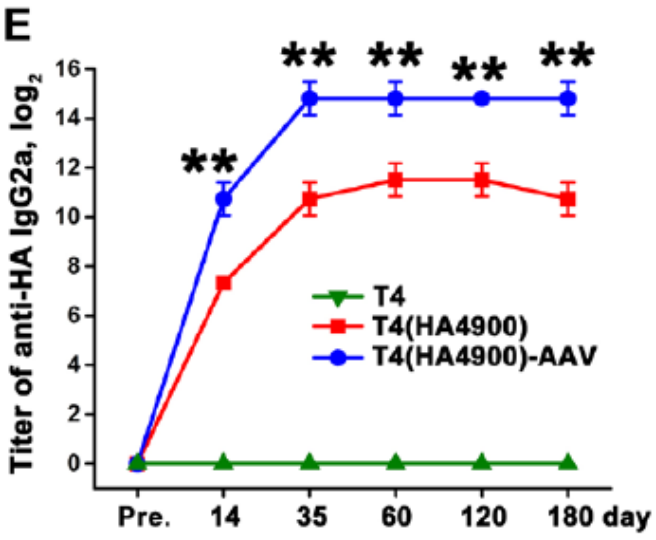
Multivalent DNA vaccine and protein antigen delivery by T4-AAV

T4 delivery vector containing the HA stem (HA4900) DNA

Antigen formulations
T4(HA4900)
T4(HA4900)-AAV(VRC)
F1mutV-T4(HA4900)
F1mutV-T4(HA4900)-AAV(VRC)
T4-AAV(HA4900)
F1mutV-T4-AAV(HA4900)



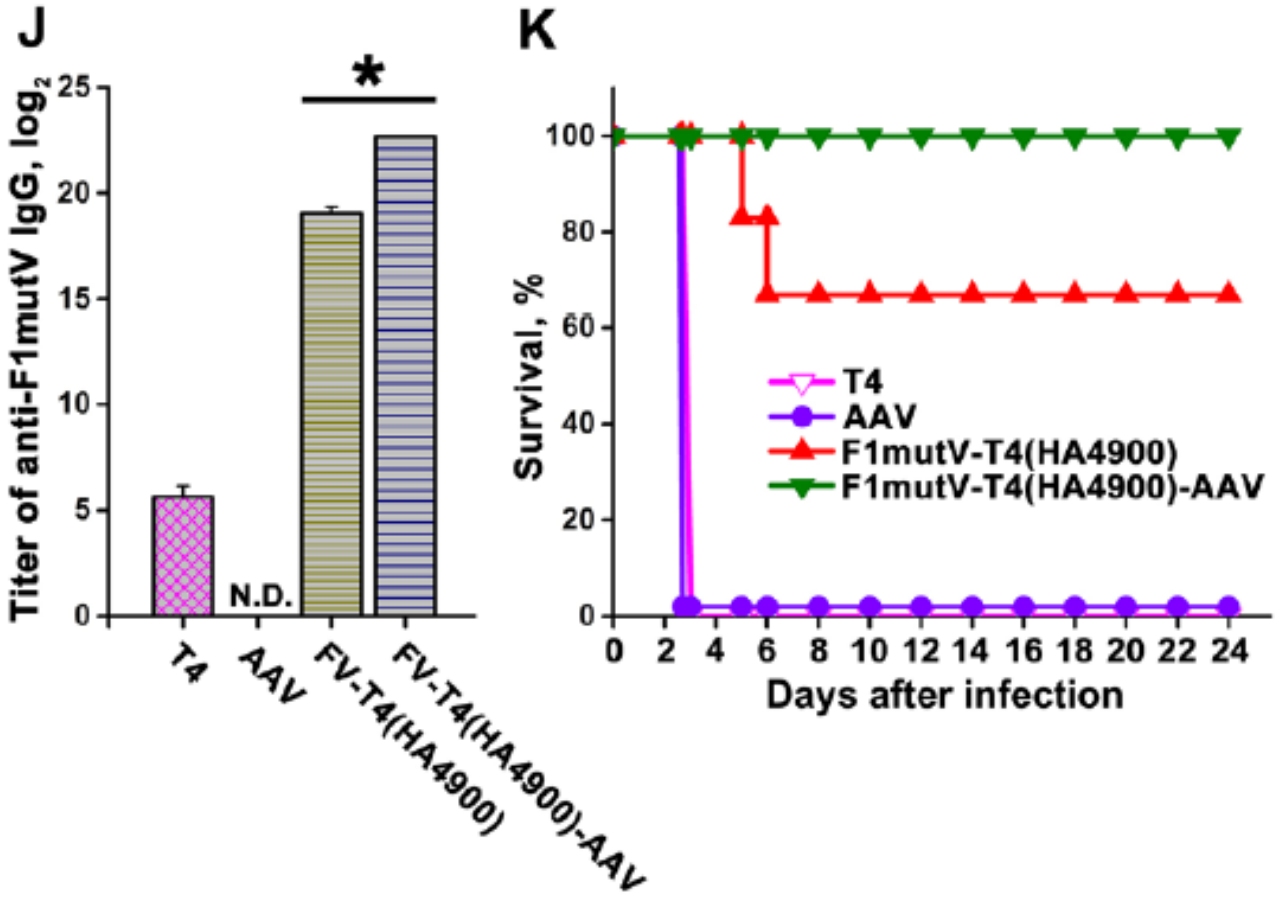




Biotinylation of Soc and Hoc

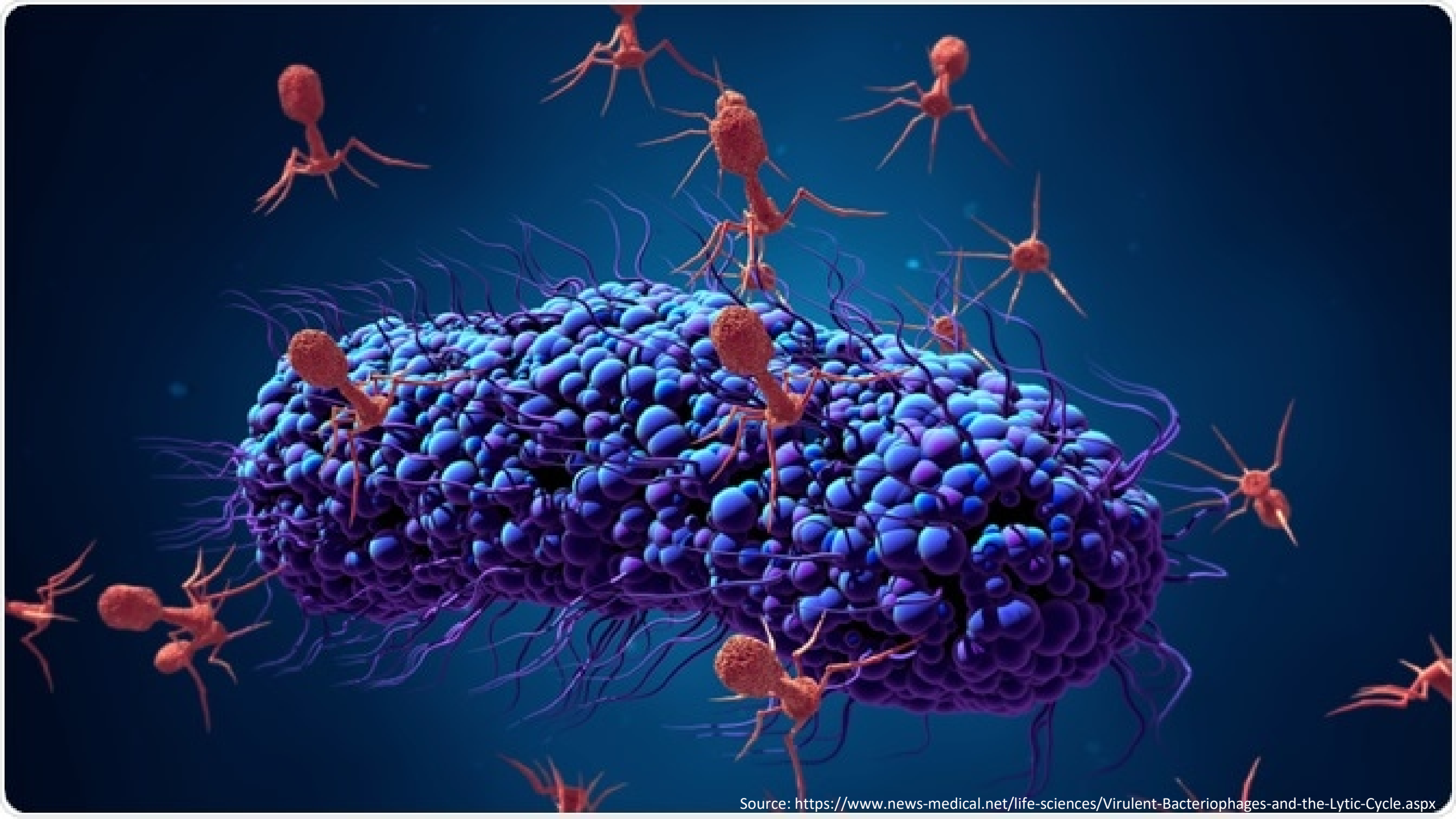
Displayed the plague antigen F1mutV on the exterior of the T4(HA4900)-AAV nanoparticles as a Soc fusion protein

Mice then were treated with lethal *Y. pestis* CO92



Conclusions II

- unique prokaryotic-eukaryotic hybrid vector that can deliver complex and large cargos of genes **and**/or proteins into mammalian cells
- The T4-AAV vector developed incorporates the useful properties of two key viruses: high-capacity, multifunctional T4 phage coupled with efficient entry and long-term gene expression by AAV.
- (potential usage for e.g. guide plasmids and donor DNA molecules as well as Cas9 as displayed proteins for efficent gene editing)
- Future hybrid vectors could have a great potential for gene therapy



Source: https://www.news-medical.net/life-sciences/Virulent-Bacteriophages-and-the-Lytic-Cycle.aspx

References:

Waehler, R., Russell, S. & Curiel, D. Engineering targeted viral vectors for gene therapy. *Nat Rev Genet* **8,** 573–587 (2007) doi:10.1038/nrg2141

Chan, K., Jang, M., Yoo, B. *et al.* Engineered AAVs for efficient noninvasive gene delivery to the central and peripheral nervous systems. *Nat Neurosci* **20,** 1172–1179 (2017). https://doi.org/10.1038/nn.4593

Jingen Zhu, Pan Tao, Marthandan Mahalingam, Jian Sha, Paul Kilgore, Ashok K. Chopra and Venigalla Rao. A prokaryotic-eukaryotic hybrid viral vector for delivery of large cargos of genes and proteins into human cells. *Science Advances* 21 Aug 2019: Vol. 5, no. 8, eaax0064. DOI: 10.1126/sciadv.aax0064