

Pretreatment with IVIG reduces peripheral transduction of AAV9 delivered to the CNS

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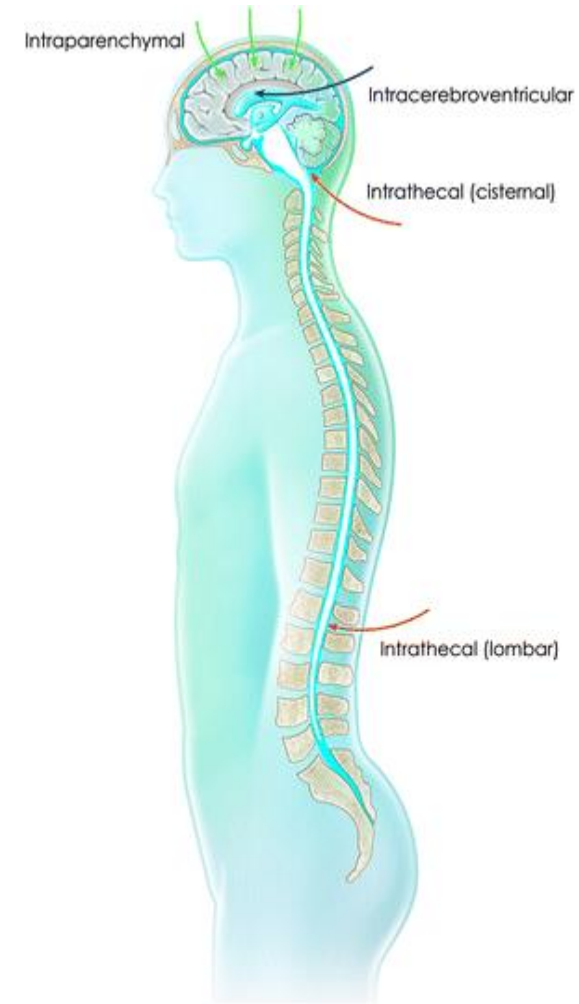
ASGCT 2022; Abs #40

Disclosures

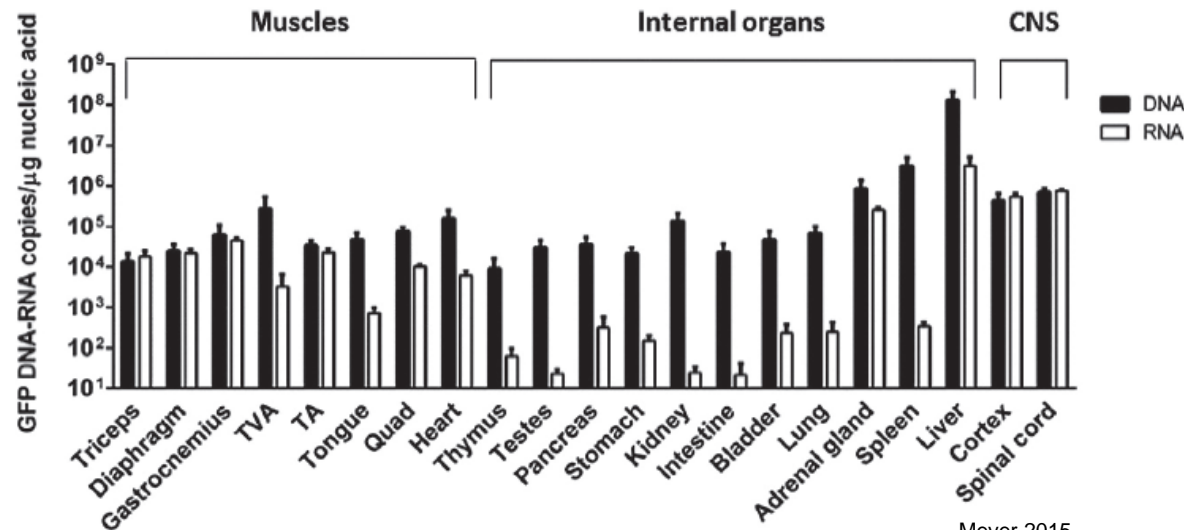
- C West, B Mastis, LK Richman, and R Calcedo are employees of Affinia Therapeutics
- LH Vandenberghe is a consultant to, holds equity in, and is a director of Affinia Therapeutics; an inventor of AAV9 and AncAAVs, for which he receives royalties; CEO of a stealth biotech, and member of the Board of Directors of Addgene and Odylia, and a constant to and Scientific Advisory Board member of Akouos, where he also owns founder shares

Off-target transduction of AAV gene therapy may limit therapeutic potential

- Following intra-CSF administration AAV vector “escapes” the CNS
 - In nonhuman primates AAV9 administered directly to CSF is present in peripheral organs, especially the liver^{1,2}
 - Severe adverse events have been reported following IV administration in AAV clinical trials including liver injury, kidney injury, cardiac insufficiency³



Intrathecal AAV9 in NHP



Meyer 2015

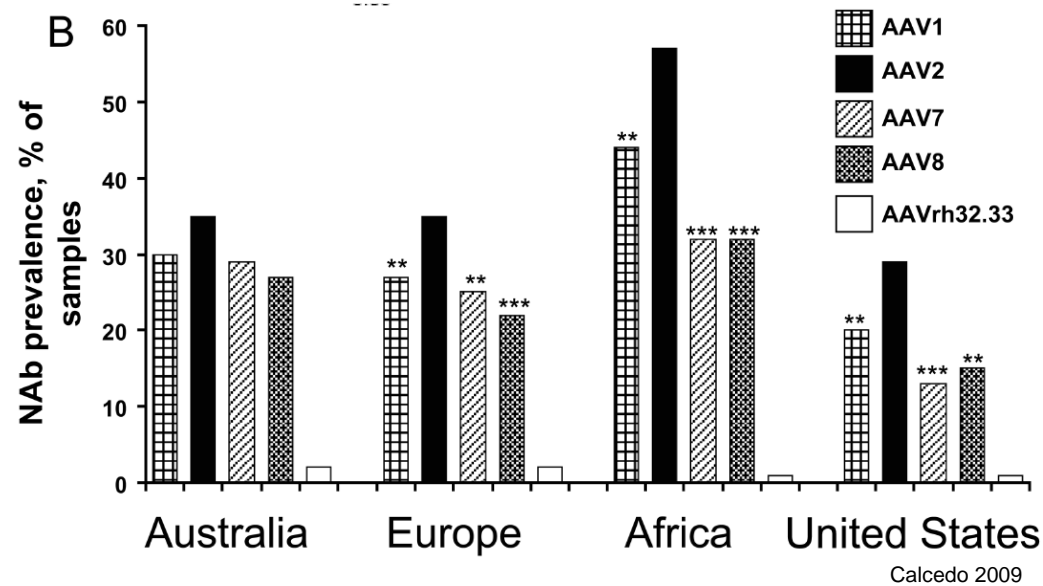
1. Meyer K et al. *Mol Ther.* 2015;23:477-87. 2. Meseck E et al. *BioRxiv.* doi.org/10.1101/2021.11.28.470258. 3. Toxicity risks of adeno-associated Virus (AAV) Vectors for Gene Therapy (GT). CTGTAC Meeting #70. Sep 2-3, 2021.

AAV: adeno-associated virus; CNS: central nervous system; CSF: cerebrospinal fluid, IV: intravenous; NHP: nonhuman primate

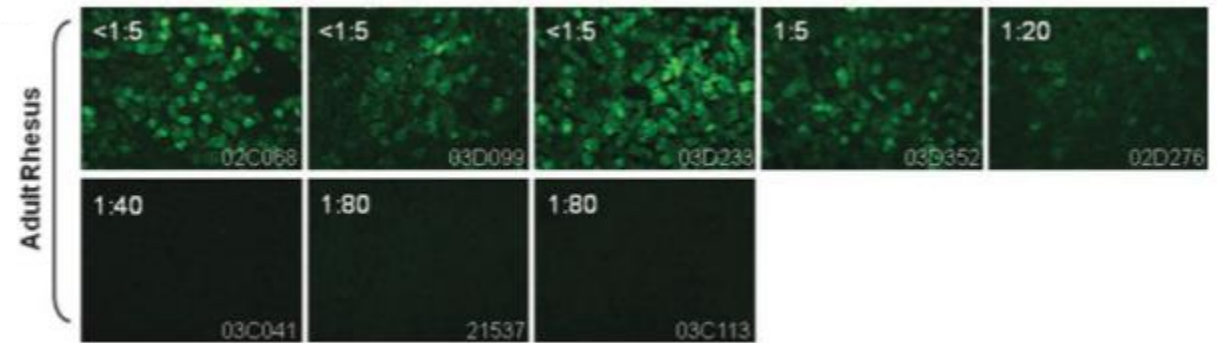
Pre-existing anti-AAV neutralizing antibodies are widespread and limit transduction

- 30-60% of population have neutralizing antibodies to given capsid¹

- Data in NHP show that higher AAV8 NAb titer results in reduced eGFP expression following IV administration²



eGFP expression in macaque liver 7 days post IV administration of AAV8.TBG.EGFP



Wang 2011

1. Calcedo R et al. *JID*. 2009;199:381-390. 2. Wang K et al. *Hum Gene Therap*. 2011;22:1389-1401.

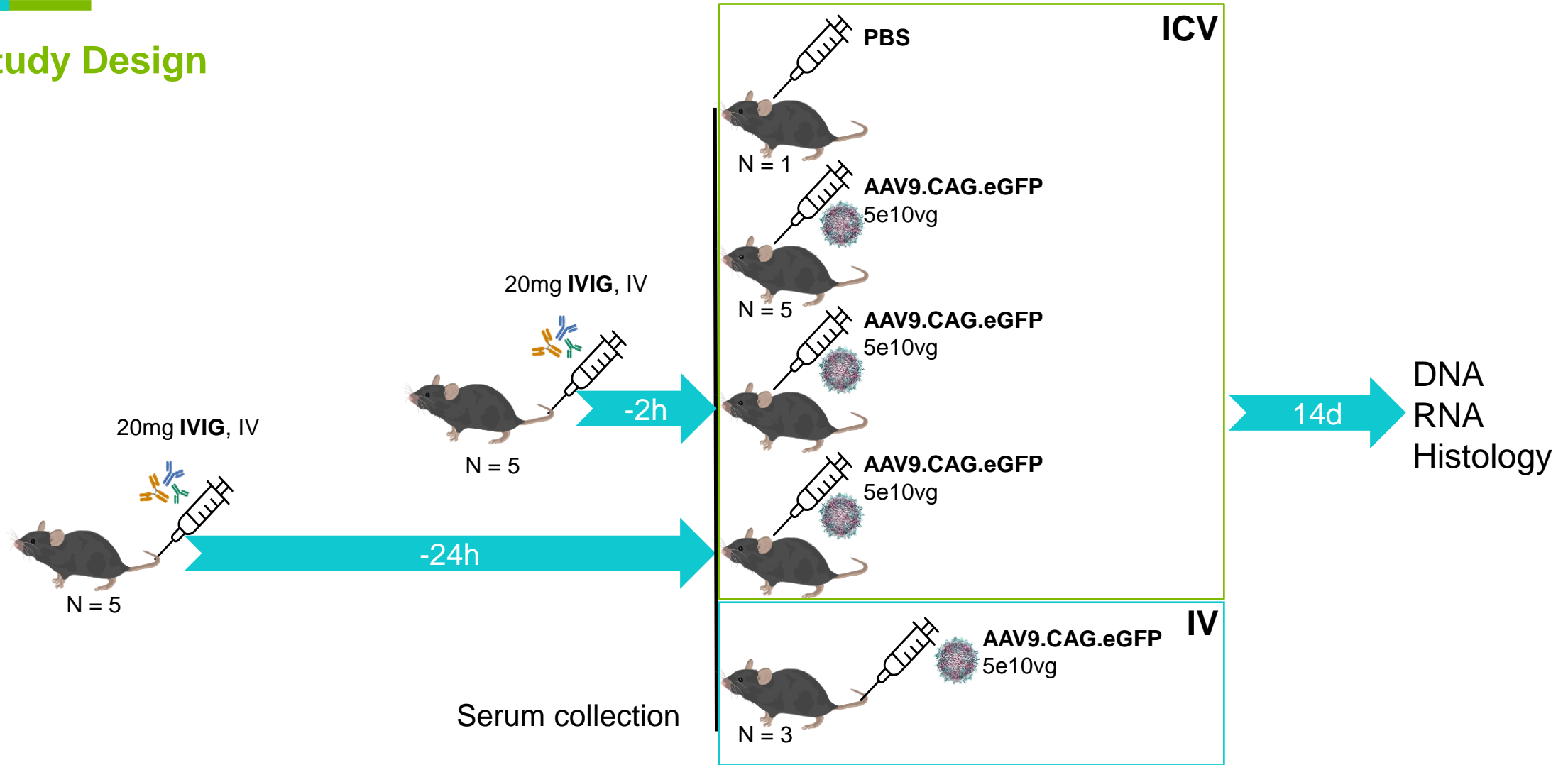
AAV: adeno-associated virus; eGFP: enhanced green fluorescent protein; IV: intravenous; NAb: neutralizing antibody; NHP: nonhuman primate; TBG: thyroxine binding globulin

Can IVIG prior to AAV administration to CNS limit transduction and expression in peripheral organs?

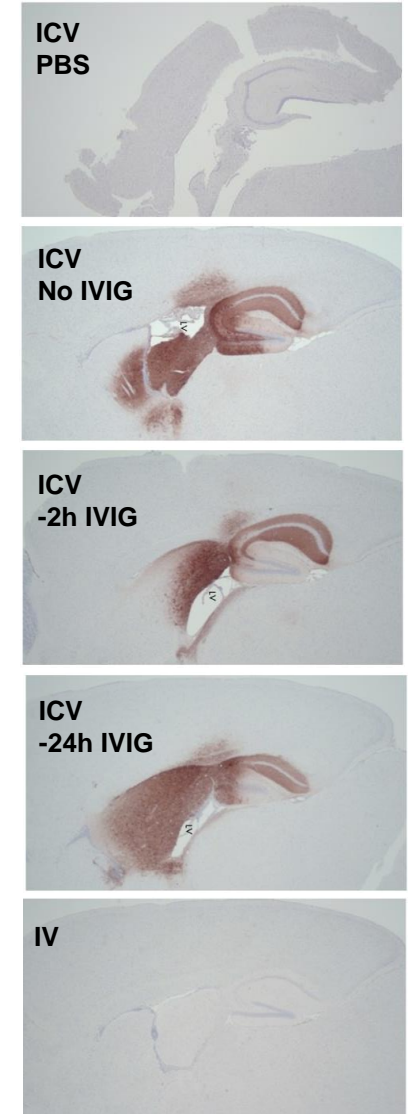
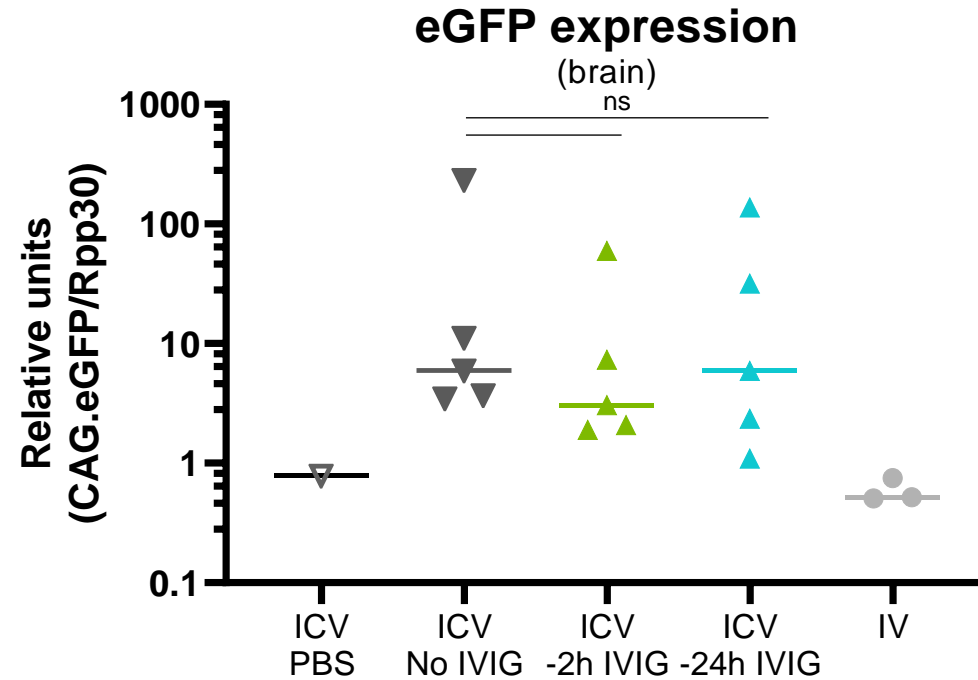
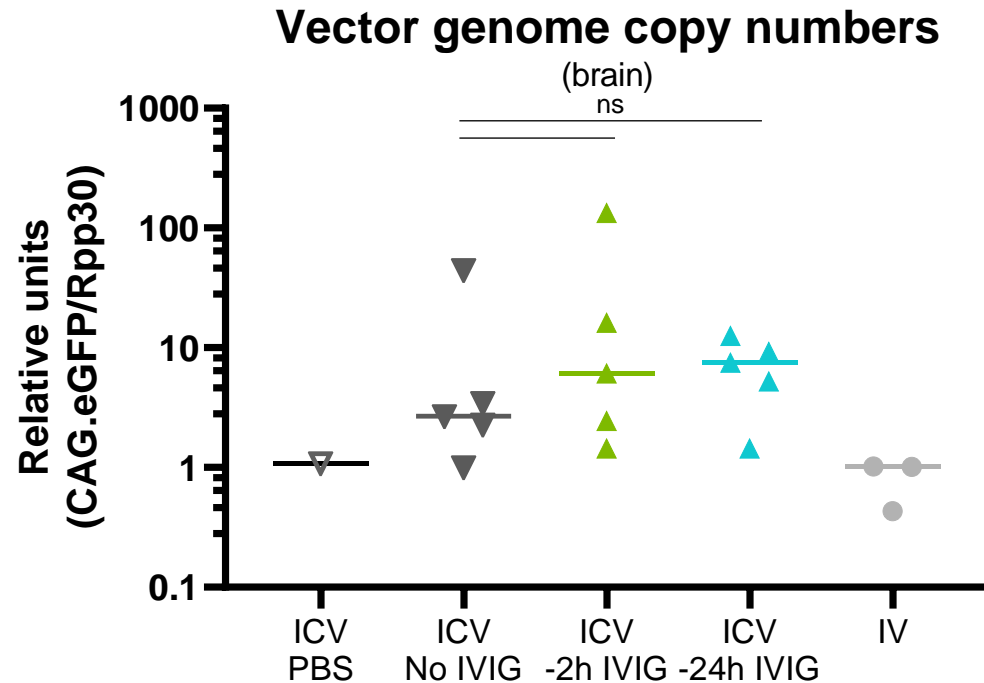
- Circulating antibodies do not readily cross the BBB
- IVIG is a widely available therapy containing antibodies against antigens present on AAV capsids
 - Derived from a large pool of donor sources and contains neutralizing antibodies to a wide spectrum of AAV serotypes

Evaluating the impact of IVIG on AAV9 vector biodistribution

Study Design

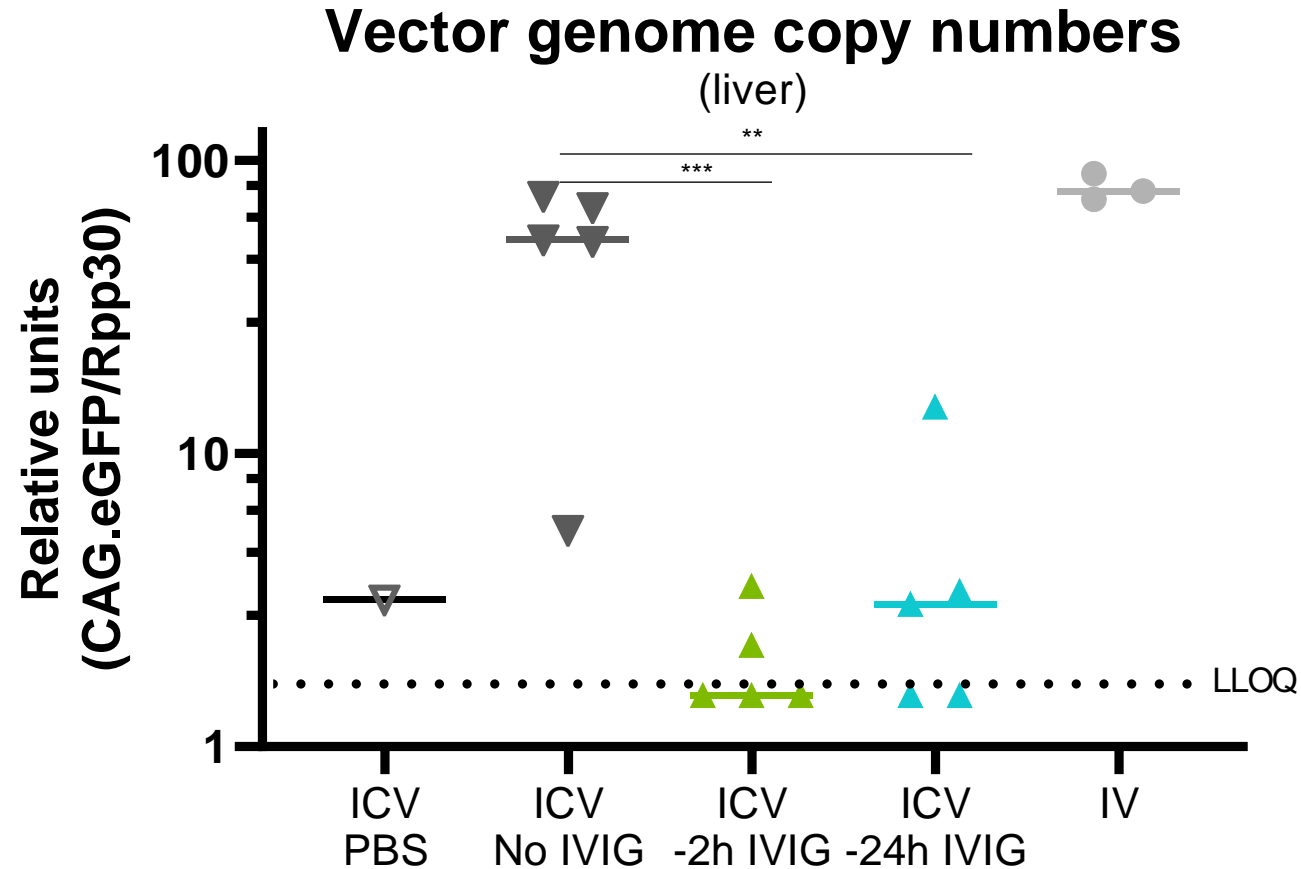


IVIG pretreatment did not affect vector transduction nor expression in brain

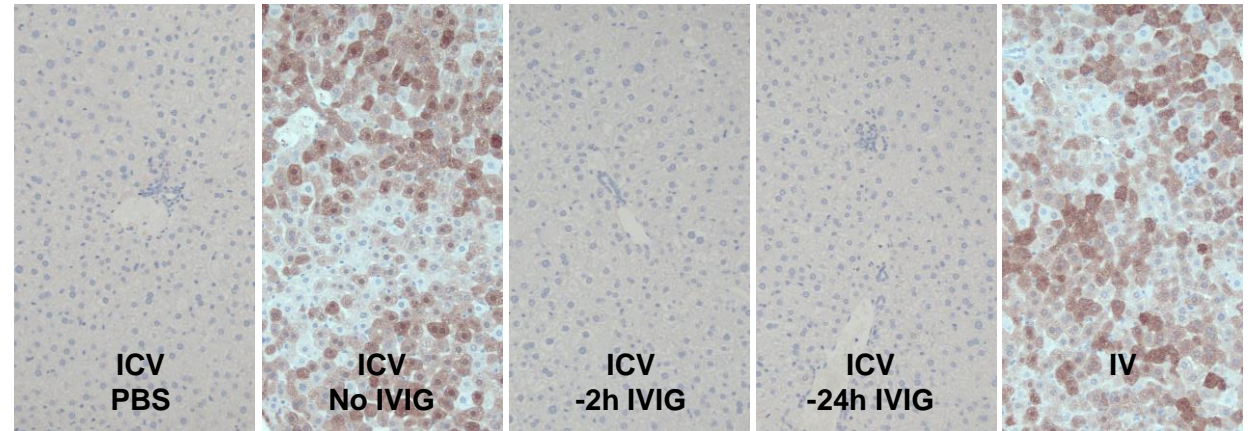
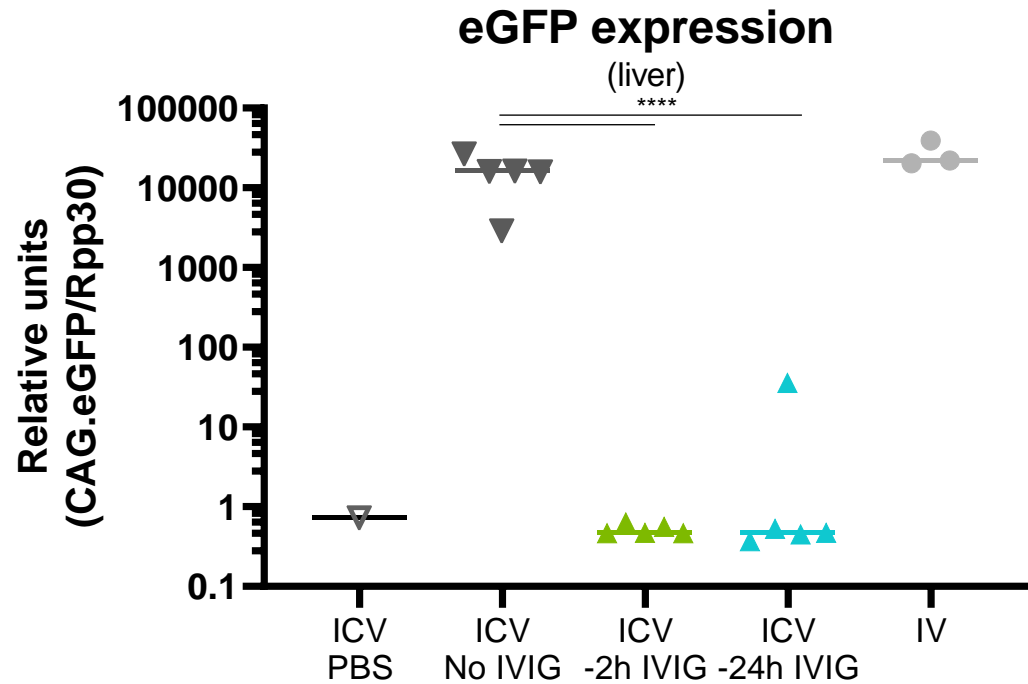


eGFP expression by IHC (brain)

IVIg pretreatment blocked vector transduction in liver

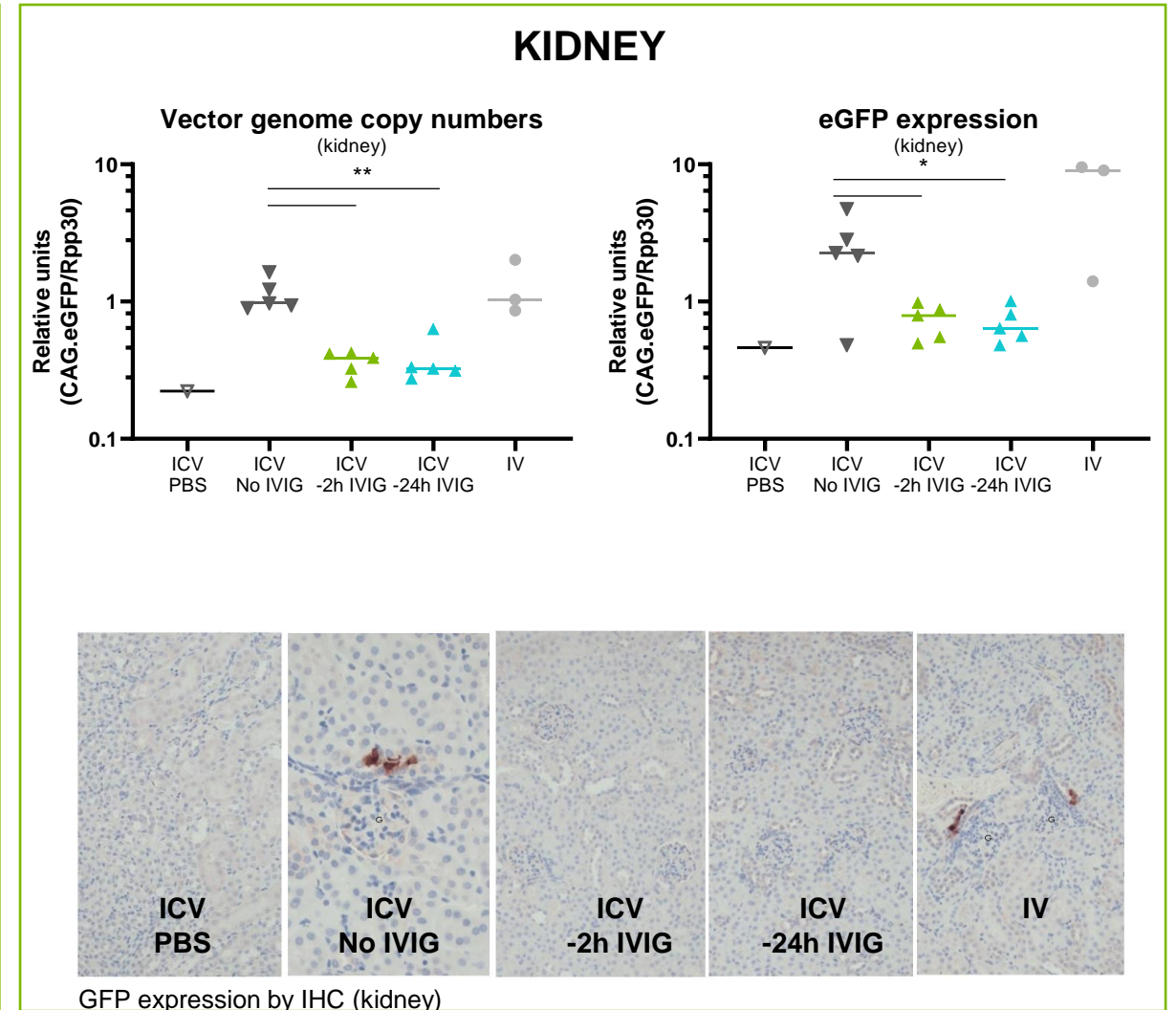
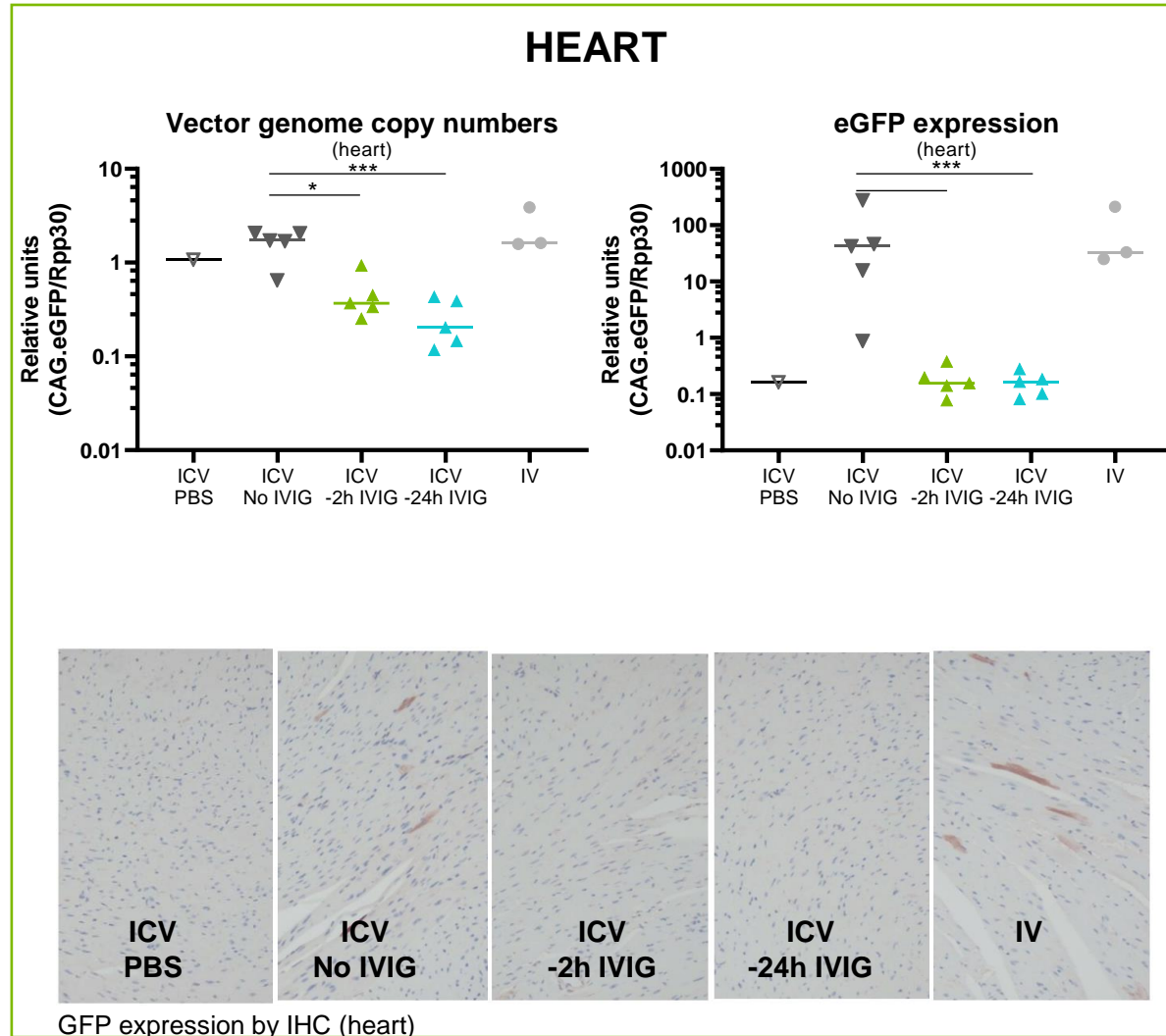


IVIG pretreatment reduced expression in liver



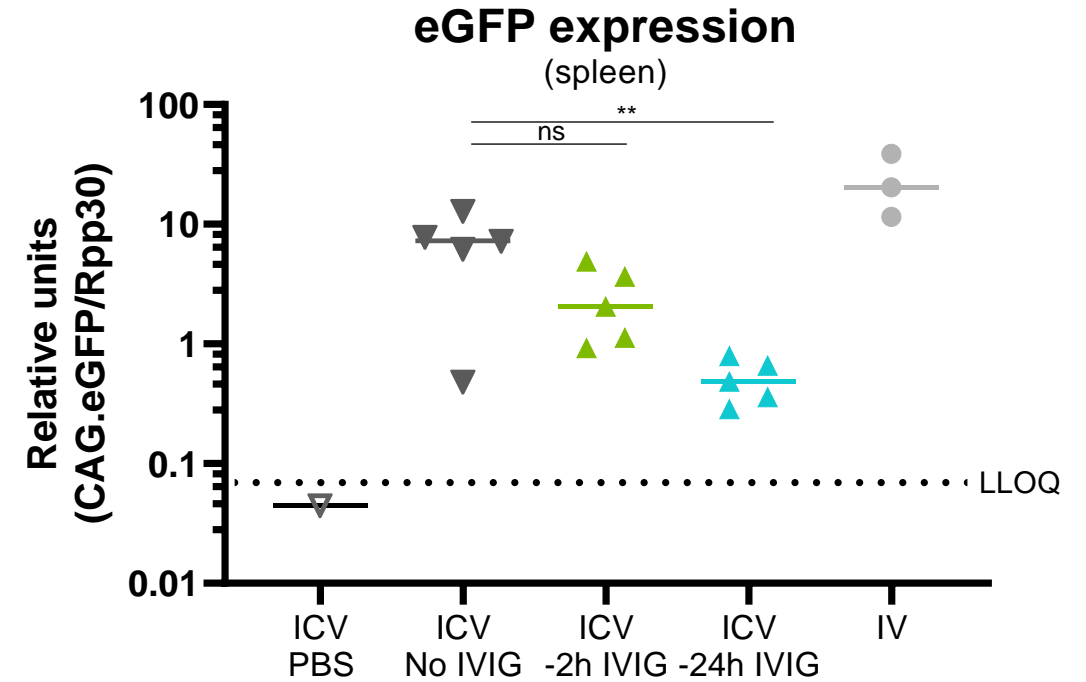
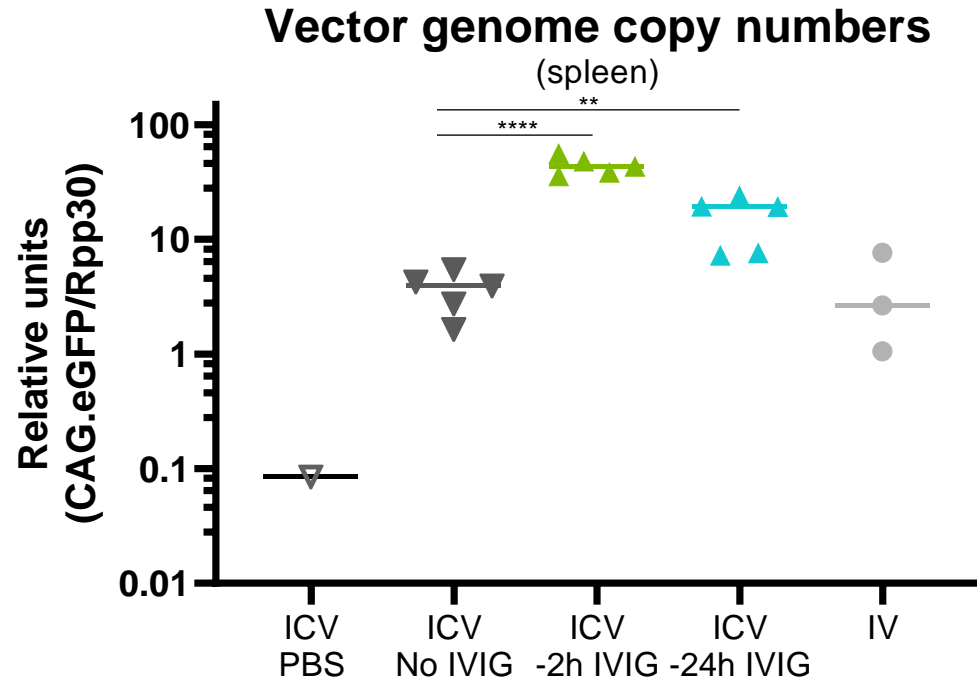
GFP expression by IHC (liver)

IVIG pretreatment blocked vector transduction and expression in additional peripheral tissues

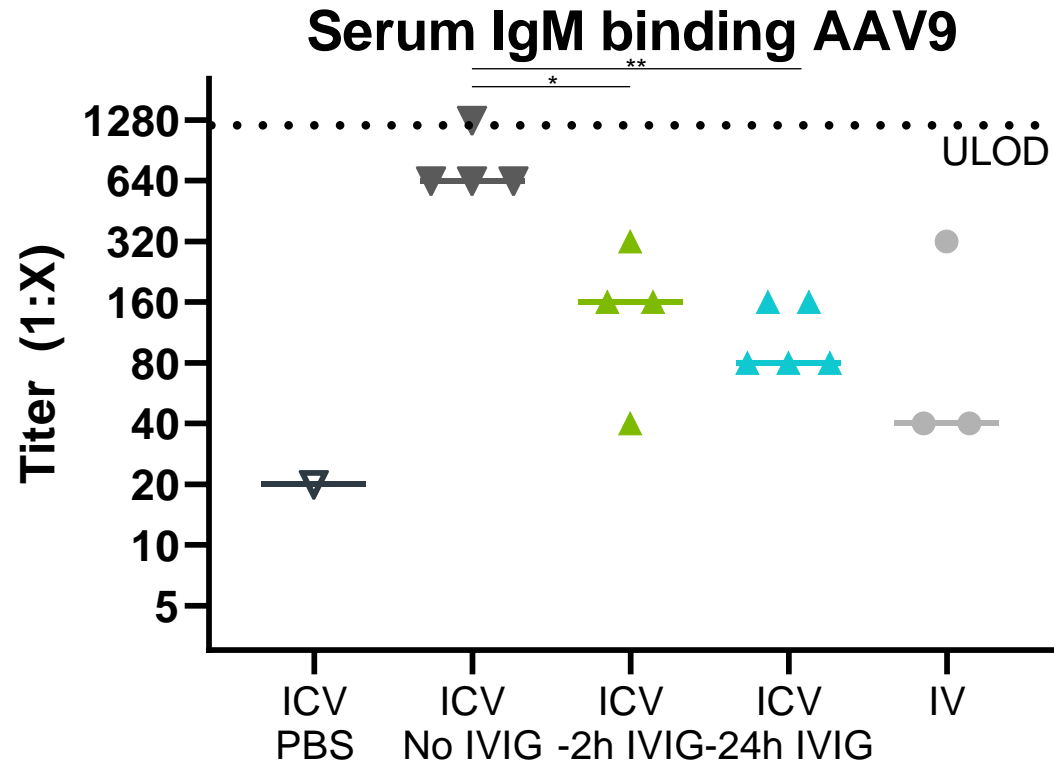


AAV: adeno-associated virus; CAG: chicken beta-Actin; eGFP: enhanced green fluorescent protein; ICV: intracerebroventricular; IHC: immunohistochemistry; IV: intravenous; IVIG: intravenous immunoglobulin; PBS: phosphate-buffered saline; RPP: ribonuclease P protein; ns: not significant, **: $p < 0.01$, *: $p < 0.02$. One-way ANOVA using Tukey's multiple comparisons test.

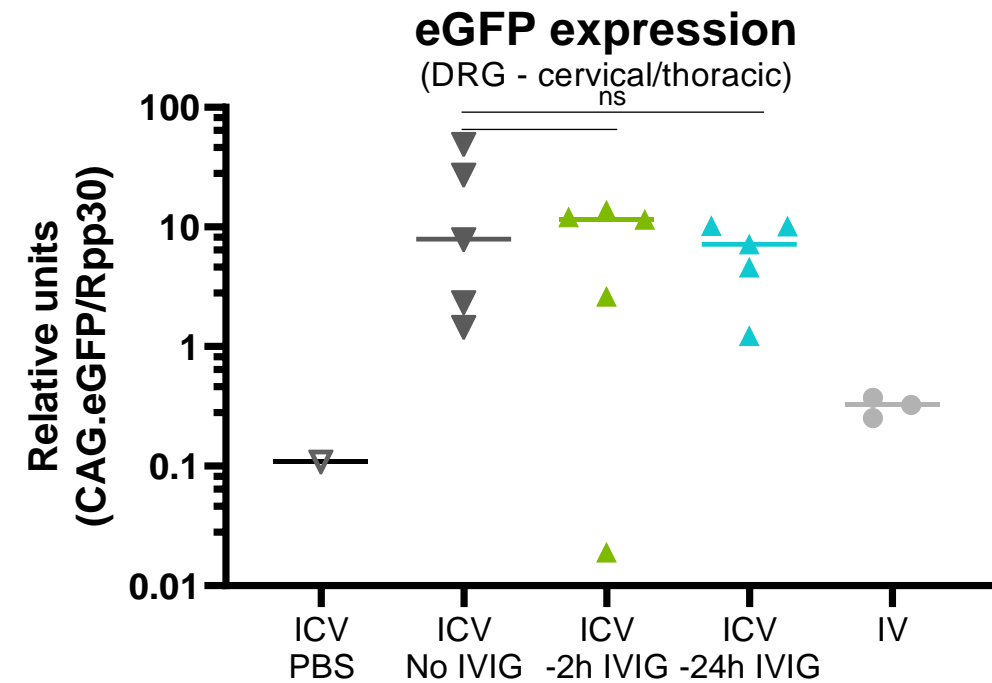
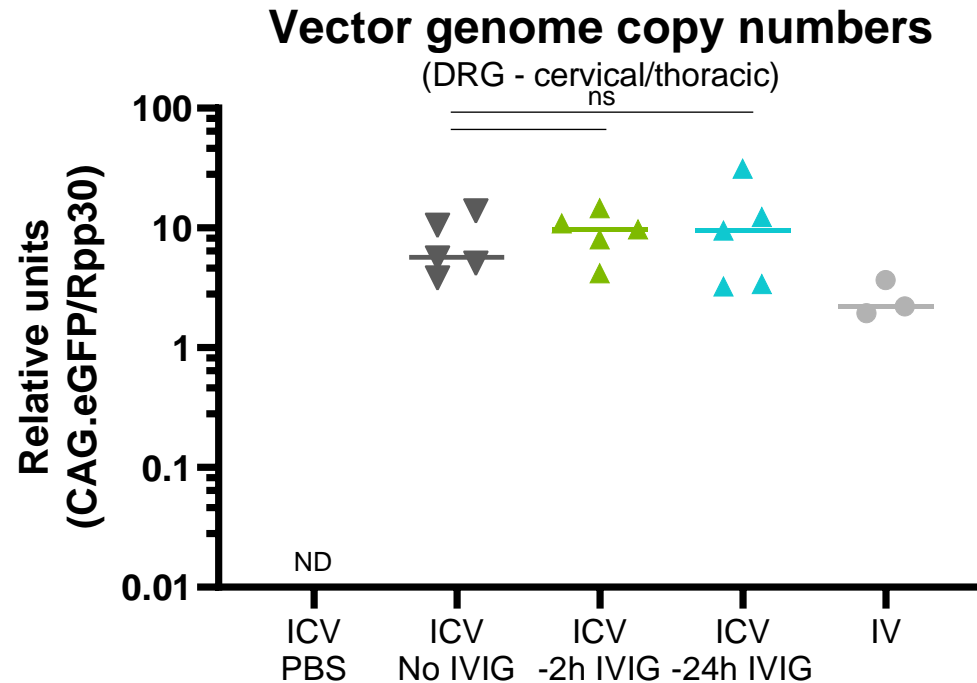
IVIG pretreatment resulted in increased AAV genome copies but no change in expression in spleen

Systemic IVIG administration prior to ICV AAV9 administration lowers murine IgM binding antibody titers



IVIg pretreatment did not affect vector transduction nor expression in DRG



ND, not detected

Intravenous immunoglobulin (IVIG) pretreatment minimizes off-target effects in periphery following CNS-delivered AAV gene therapies

- High levels of vector genomes in peripheral organs following ICV administration confirm AAV9 escapes the CNS
- Pretreatment with IVIG did not impact vector transduction nor gene expression in brain or DRG
 - Findings suggest AAV transduces brain and DRG directly from the CNS.
- Pretreatment with IVIG reduced vector transduction and gene expression in liver, heart, and kidney
- Pretreatment with IVIG increases vector genomes but lowers gene expression in spleen as vector is engulfed and degraded by phagocytes.
 - Pretreatment with IVIG results in lower humoral immune response to AAV capsid.
- Additional studies in NHPs are needed to confirm these findings

Acknowledgements

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 - Harvard Medical School

Thank you!

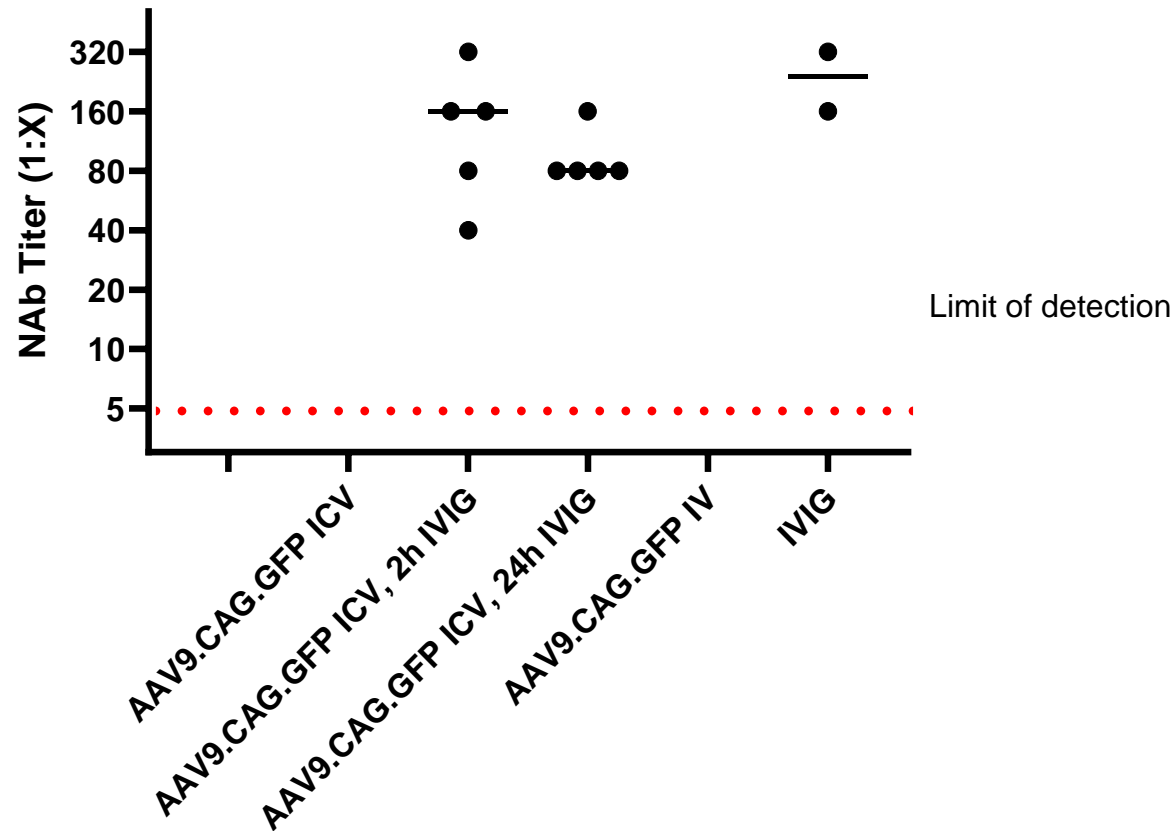




Backup slides

Animals treated with IVIG have expected NAb titers

Serum collected prior to vector administration



#	Animal ID	Sample Type	AAV9 NAb ₅₀ in HEK293 cells ^{1,2}
1	#2	Serum	< 10*
2	#3	Serum	< 5
3	#4	Serum	< 10*
4	#5	Serum	< 5
5	#6	Serum	< 5
6	#7	Serum	320
7	#8	Serum	160
8	#9	Serum	40
9	#10	Serum	80
10	#11	Serum	160
11	#12	Serum	80
12	#13	Serum	80
13	#14	Serum	80
14	#15	Serum	160
15	#16	Serum	80
16	#17	Serum	< 40**
17	#18	Serum	< 10*
18	#19	Serum	< 10*
19	IVIG aliquots 1	IVIG	160
20	IVIG aliquots 2	IVIG	320