OMTM, Volume *9*

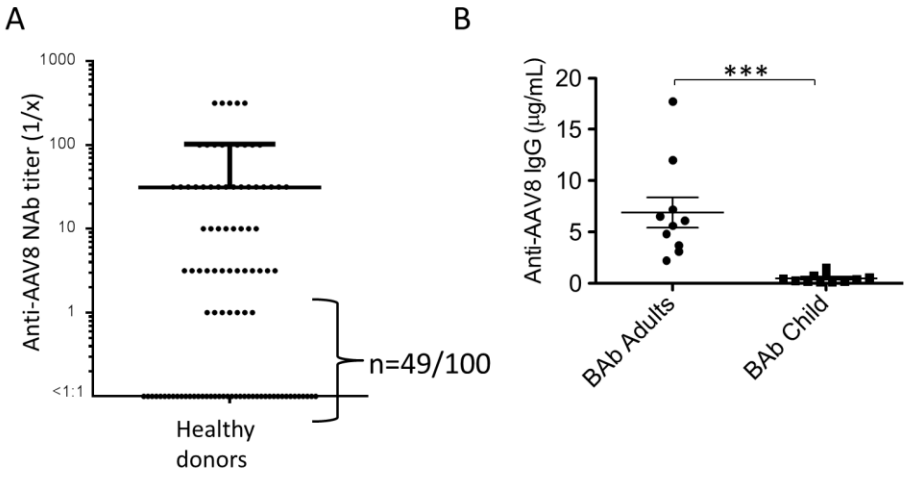
Supplemental Information

Influence of Pre-existing Anti-capsid Neutralizing and Binding Antibodies on AAV Vector Transduction

Zachary Fitzpatrick, Christian Leborgne, Elena Barbon, Elisa Masat, Giuseppe Ronzitti, Laetitia van Wittenberghe, Alban Vignaud, Fanny Collaud, Séverine Charles, Marcelo Simon Sola, Fabienne Jouen, Olivier Boyer, and Federico Mingozzi

Supplementary Data

Supplementary Figure S1



Detection of anti-AAV8 neutralizing and binding antibodies in a collection of human serum samples

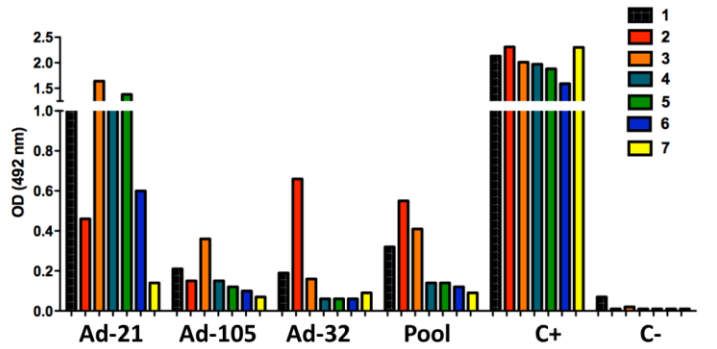
(A) Neutralizing antibody titer (NAb) specific to AAV8. The number of samples with anti-AAV8 NAb titer equal or below 1:1 (non-neutralizing, n=49/100) is indicated. (**B**) Anti-AAV8 IgG ELISA of the 49 adult subjects with NAb titer equal or below 1:1 (Bab Adults) compared with a collection of 11 serum samples from children aged ~1 year and with negative NAb titer (BAb Child). Statistical significance was determined using the Student's t-test. ***, $p<0.001$. Data are represented as mean± SEM.

Supplementary Figure S2

A

Batch #	Production method	Purification method	Capsid content
1	Transfection	Gradient	Full
2	Transfection	Gradient	Empty
3	Transfection	Gradient	Full
4	Baculovirus	Column	Full/Empty
5	Baculovirus	Column	Full/Empty
6	Transfection	Gradient	Full
7	Transfection	Gradient	Empty

B

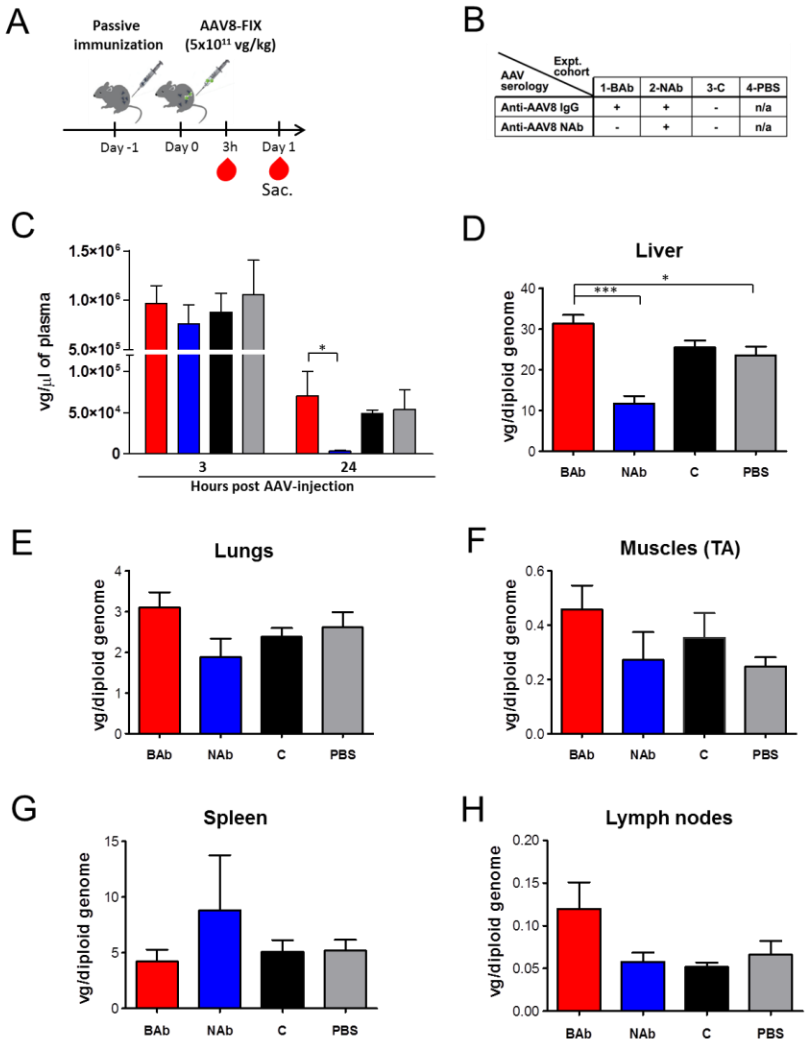


NAb titer	< 1:1	< 1:1	< 1:1	<1:1	1:3160	< 1:1

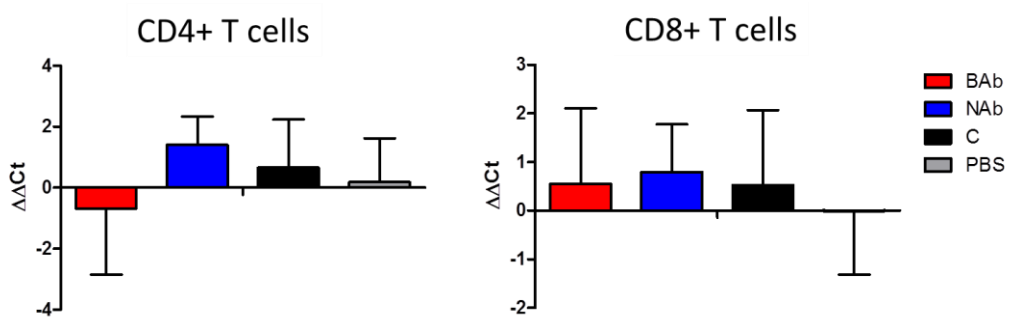
Binding of non-neutralizing anti-AAV8 capsid IgG to different AAV8 vector preparations

(A) Description of the virus batches. Transfection, AAV batches produced by triple transfection of HEK293 cells. Baculovirus, AAV batches produced baculovirus infection of SF9 insect cells. Gradient, AAV vectors purified by CsCl gradient centrifugation. Column, AAV vectors purified by affinity column chromatography. Full, AAV8 capsid containing a genome. Empty, genome-free AAV8 capsids. (B) Binding assay to detect anti-capsid IgG in serum samples derived from donors harboring non-neutralizing anti-AAV8 capsid antibodies (Ad-21, Ad-105, and Ad-32) or in a pool of sera from the same donors (Pool). C+, donor with a high NAb titer. C-, seronegative non-human primate sample. Plates were coated with the capsid preparations listed in panel A. OD, optical density. The neutralizing antibody (NAb) titers of the samples used in the binding assay is indicated below panel B.

Supplementary Figure S3



Persistence of AAV8 vector in the bloodstream of mice harboring pre-existing neutralizing or non-neutralizing antibodies and evaluation of the vector biodistribution (A) Experimental design, red symbols represent timing of blood collection. Sac., sacrifice. (B) Treatment cohorts. (C) Clearance of vector genomes from plasma over time. Vector genome copy number per diploid genome in the liver (D), lungs (E), muscles (F), spleen (G) and lymph nodes (H) of animals at the time of sacrifice. Statistical significance of BAb over the NAb, C and PBS group was determined using Student's t-test. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. Data are represented as mean± SEM. BAb, binding antibodies. NAb, neutralizing antibodies. C, children naïve to AAV8.



Supplementary Figure S4 - Detection of CD4 and CD8 T cell infiltrates in the liver of mice harboring pre-existing neutralizing or non-neutralizing antibodies

CD4 and CD8 mRNA expression levels were normalized for the level of expression of GAPDH housekeeping gene (ΔCt) and then to the average level measured in control group (ΔΔCt).