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Supplemental Information

Successful Repeated Hepatic Gene Delivery in Mice and Non-human Primates Achieved by Sequential Administration of AAV5^{ch} and AAV1

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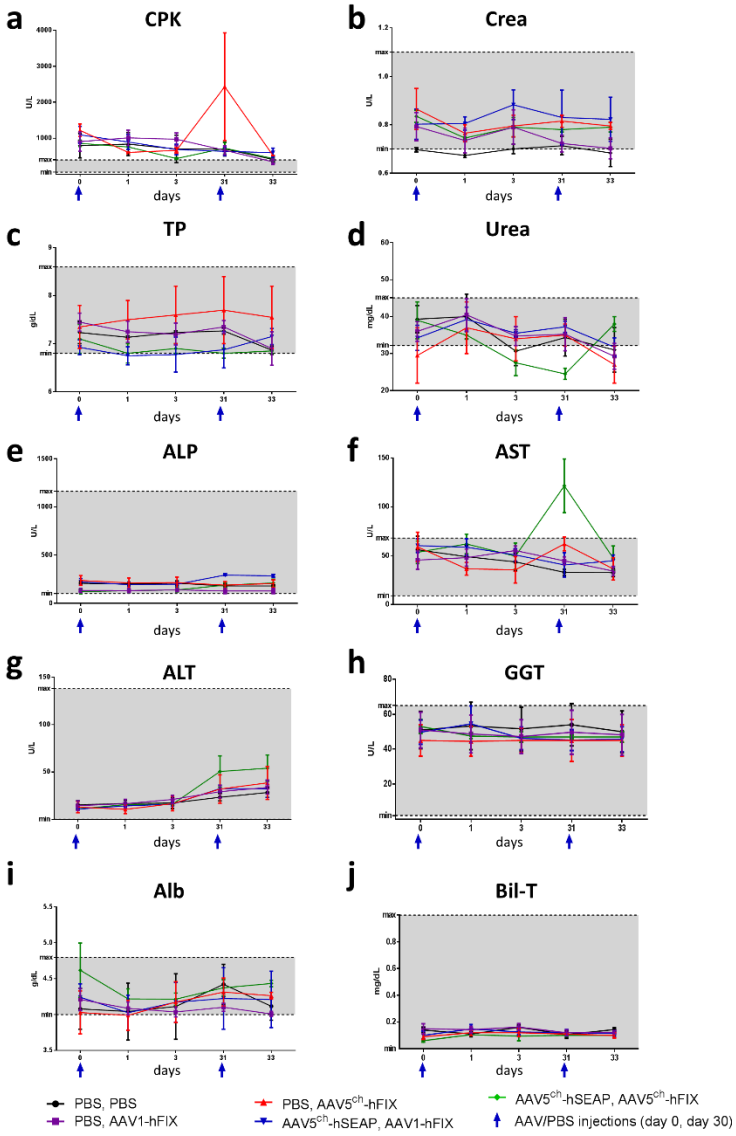
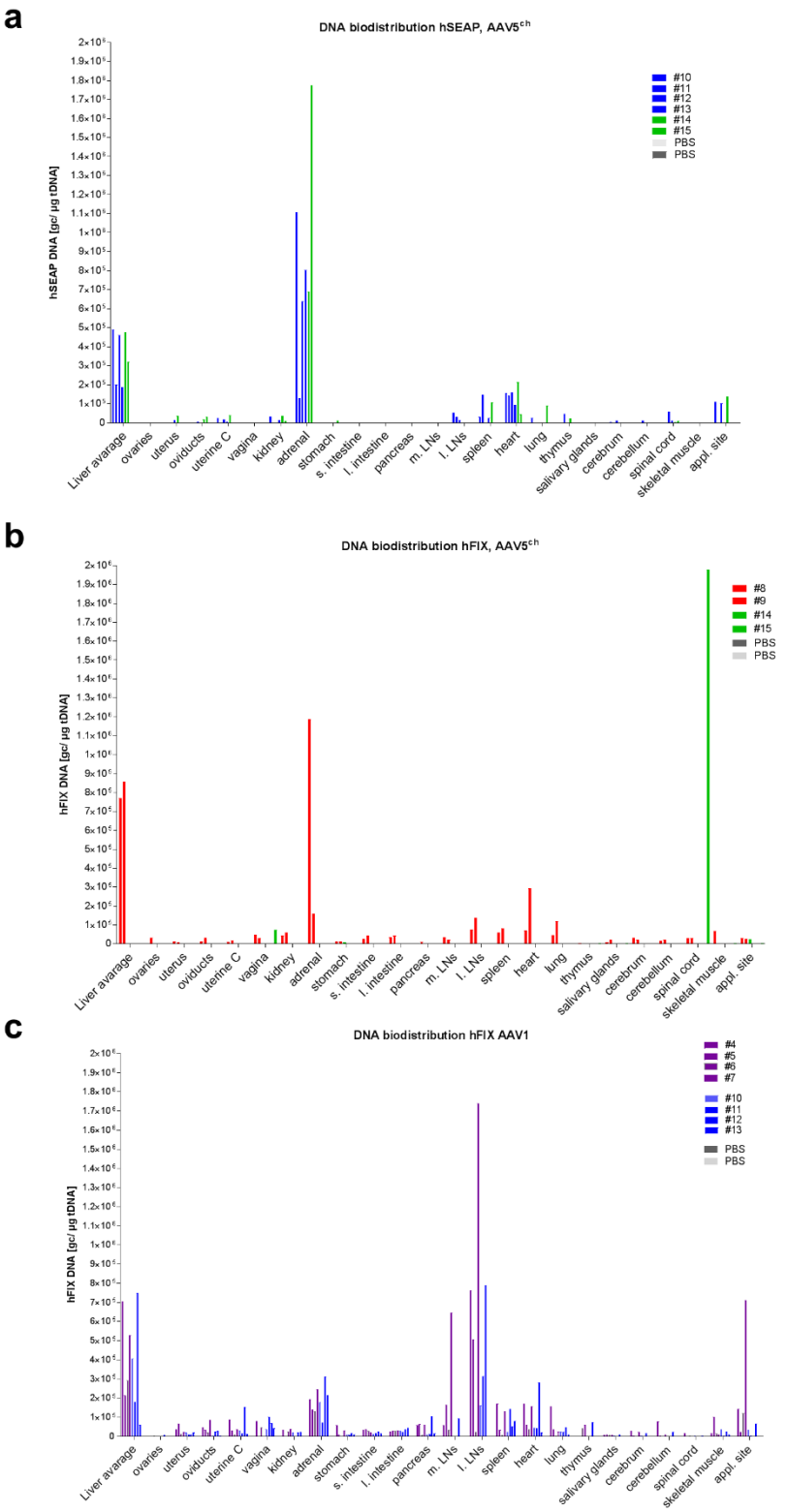


Supplementary Figure 1

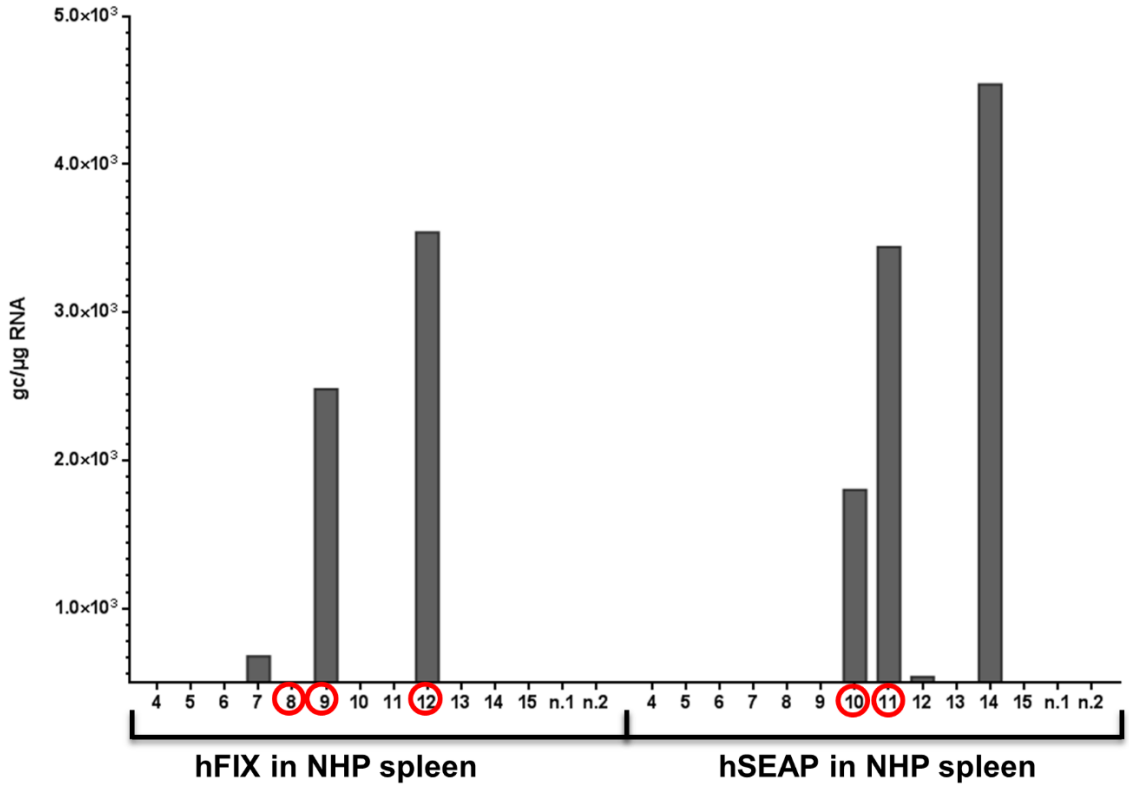
Serum biochemistry. Mean values of CPK, Crea, TP, ALP, AST, ALT, GGT Alb and bil-T at different time points around time of AAV vector administrations (day 0 and day 30). The horizontal dashed lines represent the upper and lower normal values for the different parameters in Macaca fascicularis. CPK- Creatine Phosphokinase (a), Crea- creatinine (b), TP- total protein (c), urea (d), ALP- alkaline phosphatase (e), AST- aspartate aminotransferase (f), ALT- alanine aminotransferase (g), GGT- gama-glutamyl transferase (h), Alb- albumin (i) and Bil-T- total bilirubin (j).

No alterations in weight gain or food intake were reported of the treated animals when compared with the control group. Furthermore, body temperature, activity, general health and skin and mucosa color, were monitored during the course of the experiment and were found normal. Clinical laboratory parameters, including hematological parameters, coagulation tests, urine analysis, renal and liver function tests, and complete blood count, remained in the normal ranges after injections. In the 1 year period, only two observations were reported. A transient elevation of aspartate aminotransferase (AST) was observed after the second AAV injection of the 2 NHPs that received twice the same AAV5^{ch} serotype (NHP 14 and 15). This elevation was concomitant with a non-significant increase of ALT and both parameters returned to basal level after 72 hours. Also, a transient elevation of creatine phosphokinase (CPK) was observed in one out of two animals injected with AAV5^{ch} alone (NHP 9, 3930 units/L) 24h after injection. It has to be noticed that no elevation of pro-inflammatory cytokines such as IL-1β, IL-2, IL-4 at 24h or 72h after first or second injection of AAV5^{ch} or AAV1 was observed in any of the animals.



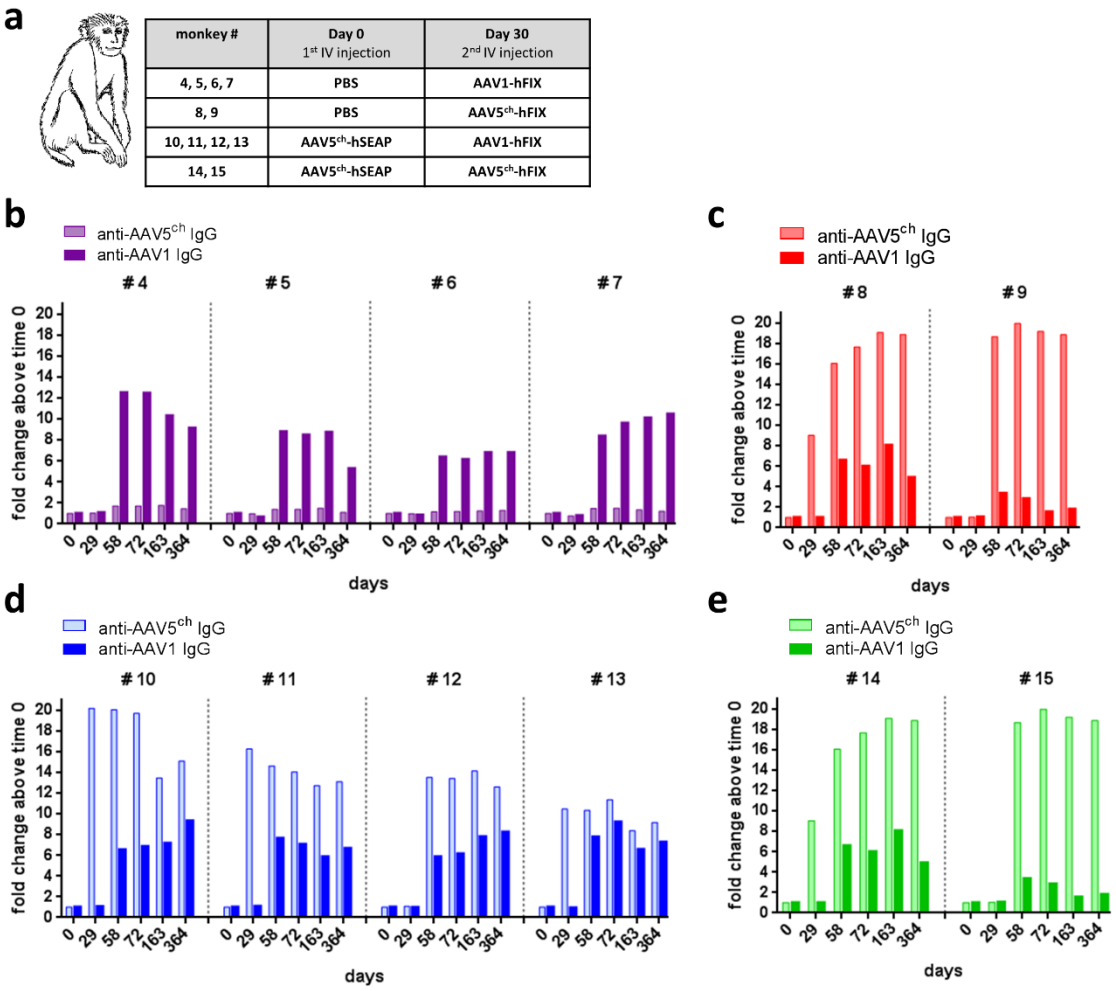
Supplementary Figure 2

Tissue biodistribution of hSEAP vector DNA after injection with AAV5^{ch}-hSEAP (a), hFIX vector DNA after injection with AAV5^{ch}-hFIX (b) and hFIX after injection with AAV1-hFIX (c) at sacrifice. Monkeys were injected iv at day 0 with an chimeric AAV5^{ch}-hSEAP (#10-15) or nothing (n.1, n.2) and at day 30 with AAV1-hFIX (#4-6 and #10-13) or AAV5^{ch}-hFIX (#14 and #15) and sacrificed at day 364.



Supplementary Figure 3

Reporter gene mRNA expression in monkey spleens. Animals that are circled in red had a decrease (NHP 8, 9) or total loss (NHP 10, 11, 12) of transgene expression during the study. Monkeys were injected iv on day 0 with an AAV5^{ch}-based vector (AAV5^{ch}-hSEAP) or PBS and on day 30 with either an AAV1- or AAV5^{ch}-based vector (AAV1-hFIX or AAV5^{ch}-hFIX).



Supplementary Figure 4

Experimental setup (a) and total anti-AAV5^{ch} anti-AAV1 IgG (b, c, d, e) levels in monkey plasma on days 0, 29, 58, 72, 163 and 364. Monkeys were injected iv at day 0 with an AAV5^{ch}-based vector (AAV5^{ch}-hSEAP) or PBS and on day 30 with either an AAV1- or AAV5^{ch}-based vector (AAV1-hFIX or AAV5^{ch}-hFIX).