OMTN, Volume *14*

Supplemental Information

Viral Vector-Based Delivery of CRISPR/Cas9 and Donor DNA for Homology-Directed Repair in an *In Vitro* Model for Canine Hemophilia B

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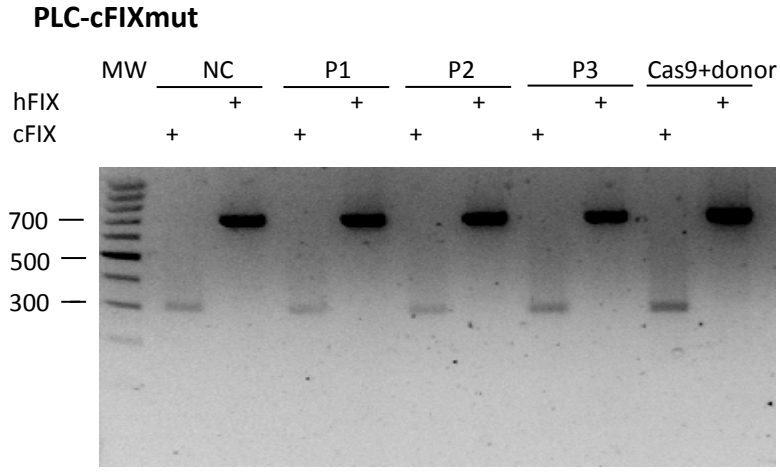
A



B

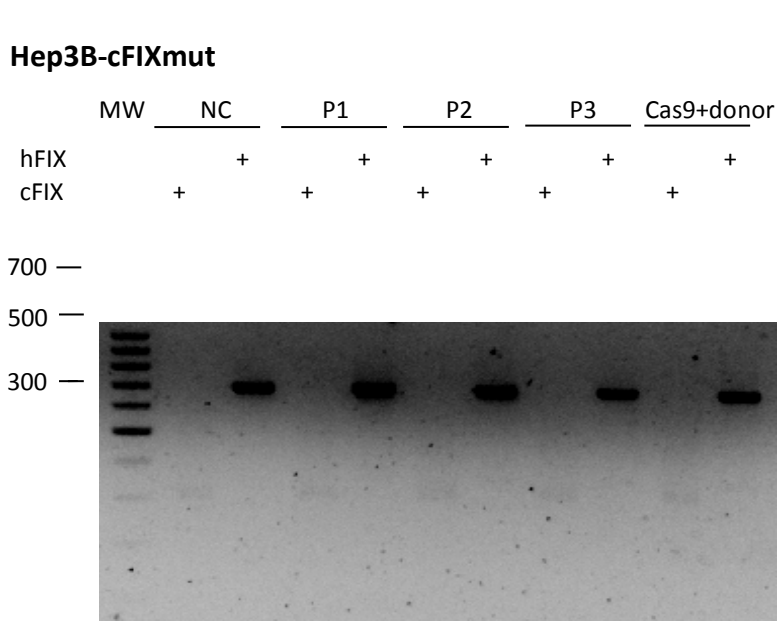


Figure S1. Indels at the uncorrected Cas9 cleavage site (no HDR occurred) in the integrated canine FIX transgene and the endogenous hFIX sequence measured by T7E1 assay after transfection of the non-viral homology directed repair plasmids. MW, molecular weight markers. NC, negative control, PLC-cFIXmut or Hep3B-cFIXmut without treatment. P1, P2, P3 and Cas9+donor mean cells treated with plasmid 1, plasmid 2, plasmid 3 or Cas9+donor.

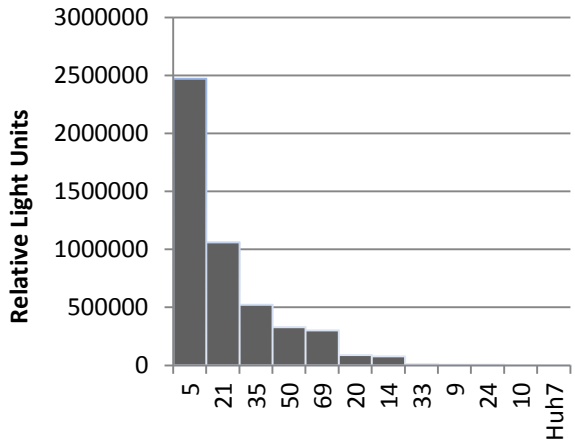
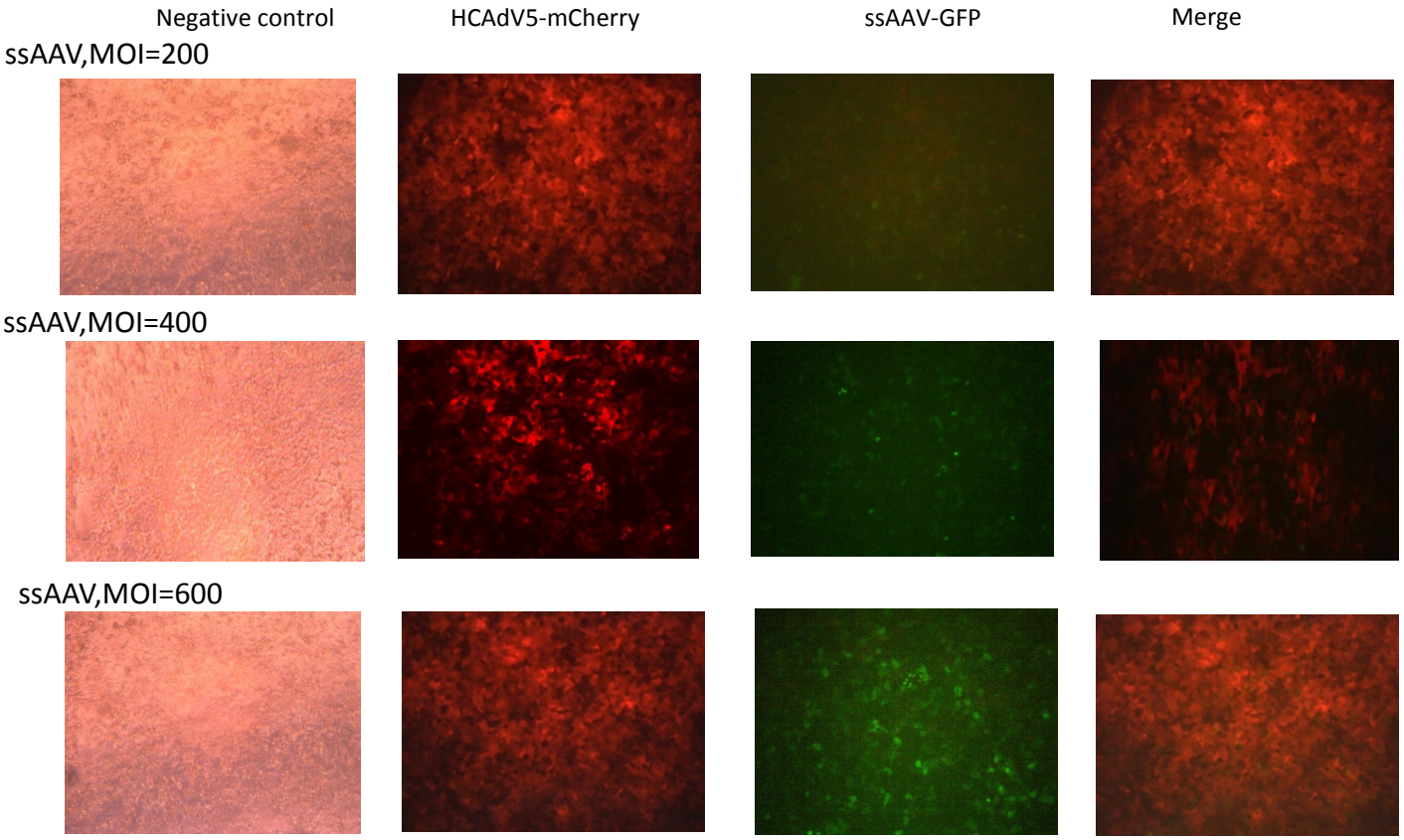


Figure S2. Liver cell infection efficiencies of different human adenovirus types. Lieber cells were transduced with luciferase tagged adenovirus types (5, 21, 35, 50, 69, 20, 14, 33, 9, 24, and 10) and luciferase values (relative light units) were measured 26 hrs post-infection.

A



B

HCAdV5-Cas9	Virus titer
Transducing units on Huh7-cFIXmut cells	8,08E+04/µl
Virus particles	9,39 E+05/µl
Negative control	undetectable

C

ssAAV2-cFIX	Virus titer
Transducing units on Huh7-cFIXmut cells	1,93E+05/µl
Virus particles	2,97E+06/µl
Negative control	undetectable

D

HCAdV5-Cas9-donor	Virus titer
Transducing units on Huh7-cFIXmut cells	1,12E+04/µl
Virus particles	2,15 E+05/µl
Negative control	undetectable

Figure S3. HCAdV5 and ssAAV transduction efficiencies in Huh7-cFIXmut cells. (A) Co-transduction of HCAdV5-GFP and ssAAV2-cherry into Huh7-cFIXmut. The Hhuh7-cFIXmut cells were co-transduced with HCAdV5-GFP (MOI=100) and ssAAV2-cherry (MOI=200, 400, 600), and the fluorescence was measured 48 hours post-transduction. **(B)** HCAdV5-Cas9 transduction efficiencies in Huh7-cFIXmut cells. **(C)** ssAAV2-cFIX transduction efficiencies in Huh7-cFIXmut cells. **(D)** HCAdV5-Cas9-donor transduction efficiencies in Huh7-cFIXmut cells. Negative control: non-infected cells. Before the conduction of HDR, the virus particle numbers and transduction efficiencies of HCAdV5-CRISPR/Cas9 and ssAAV2-cFIX on Huh7-cFIXmut cells were measured. Immediately after adding viral vectors and 3 hours post-transduction of Huh7-cFIXmut cells, total DNA was isolated and virus genome copy numbers measured using a quantitative qPCR approach. Thereby, virus particle numbers and transducing units (3 hrs post-infection) can be determined and the percentage of

transducing units per volume virus preparation can be calculated. After determining the ratio of virus particle numbers to transducing units we found that 8.6% of purified HCAdV5-Cas9 particles, 6.4% of HCAdV5-Cas9-donor particles, and 6.51% of ssAAV2-cFIX particles were active in transducing Huh7-cFIXmut cells.

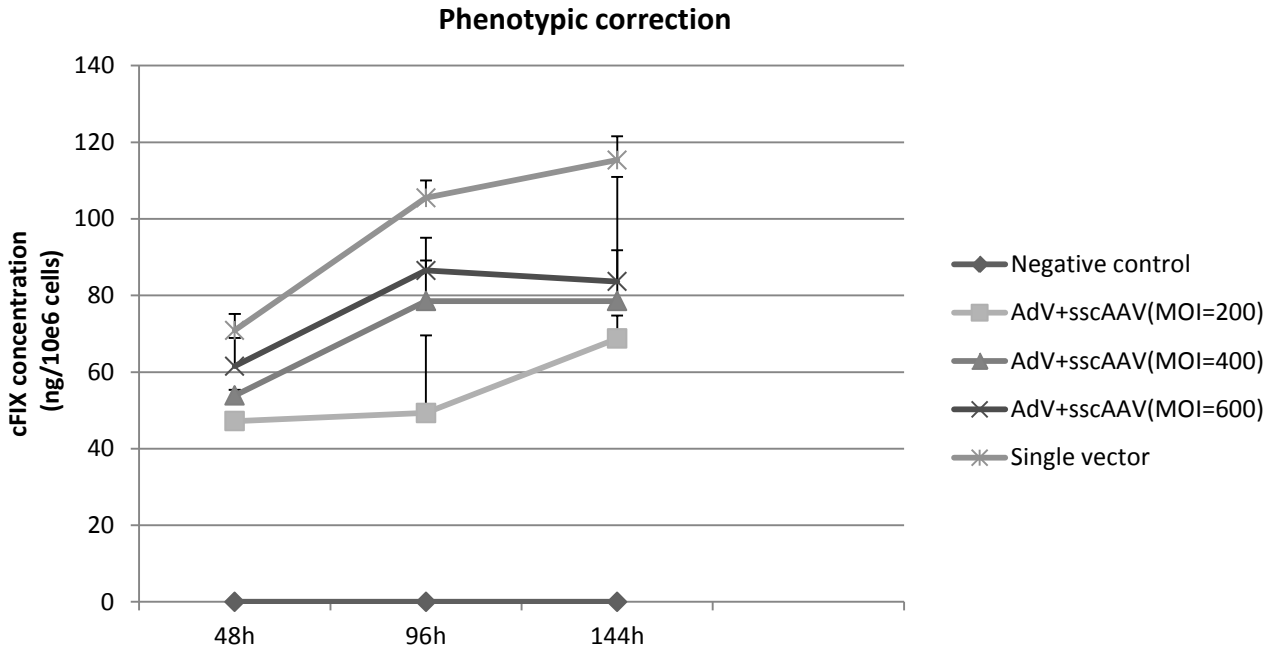


Figure S4. Genotypic and phenotypic correction in Huh7-cFIXmut cells after co-infection with the gene correction vectors HCAdV5-Cas9-cFIX or HCAdV5-Cas9 and ssAAV2-cFIX, respectively. Cellular supernatants from 24-well plates were collected at 48 hours, 96 hours, 144 hours post-transduction and analyzed by ELISA assay. ELISA assay of cFIX concentrations in the supernatant of Huh7-cFIXmut cells infected with HCAdV5-Cas9-cFIX or HCAdV-Cas9 and MOIS 200, 400 and 600 for ssAAV-cFIX. This figure correlates to **Fig. 6C**.