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A Single Administration of AAV5-hFIX in Newborn Juvenile and Adult Mice Leads to Stable hFIX Expression up to 18 Months after Dosing.

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Disclosure

All authors are employees of uniQure, shareholders of uniQure or paid consultants to uniQure

How long can AAV-mediated gene expression last?

Restoring Factor IX protein expression through gene replacement is one of the most promising treatment approaches for hemophilia B

- Single administration leads to therapeutic FIX levels and correction of bleeding phenotype[1,2]
- Constant protein expression, hence much lower risk for break-through bleeds
- We have reported years of continuous expression using AAV5-hFIX (AMT-060)[2,3]
- But how long can a non-integrative gene therapy be effective if the target cells have a high turnover?

1 Niemeyer et al 2009
2 Leebeek et al 2020
3 Miesbach et al 2018

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Can a non-integrative (AAV) gene therapy result in long-term efficacy when administered in juveniles?

AAV transgene DNA persists mainly as episomal concatamers[4,5]

Will transgene DNA loss occur due to liver growth or natural turnover of cell, leading to a loss of the therapeutic effect?

Objective of the current study:

To investigate long term sustainability of hFIX vector DNA in the liver and hFIX expression over time and during significant (liver) growth

4 Duan et al 1998
5 Salganik et al 2015

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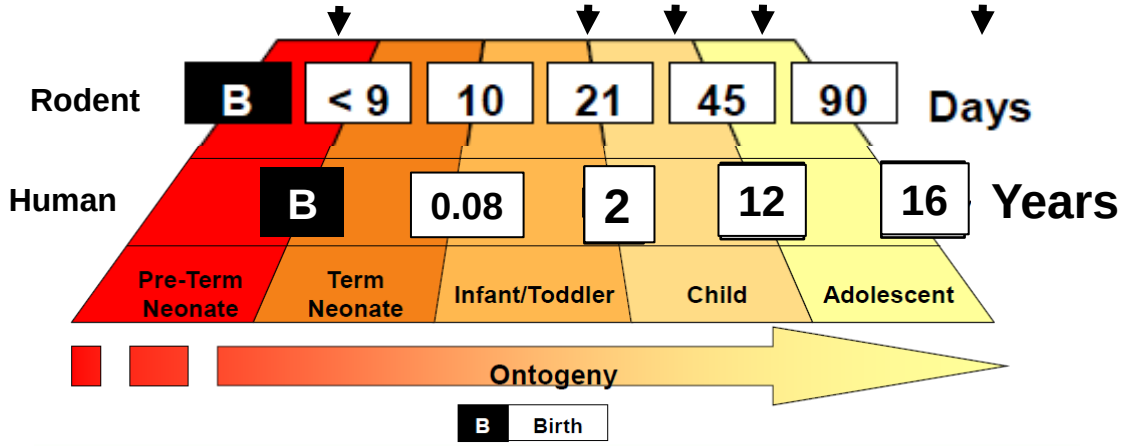
Study design:
Single high dose AAV5-hFIX injection in mice at different ages

Male C57Bl/6 mice were intravenously injected with AAV5-hFIX

Group	Age at dosing	AAV5-hFIX Dose (gc/kg)	Mean BW at dosing (gram)	Mean total gc/animal
1	2 days	2.3 x 10^{14}	1.7 g	$4.0x10^{11}$
2	3 weeks		9.8 g	$2.3x10^{12}$
3	6 weeks		20.9 g	$4.8x10^{12}$
4	11 weeks		31.9 g	$7.4x10^{12}$
5	6 months		37.1 g	$8.5x10^{12}$

a) Each AAV5-hFIX dose group had an age matched control group dosed with vehicle



Modified from Buelke-Sam 2001

Age groups were selected to present a range of ages and growth rates

Necropsy of all groups at 18 months of age – interim at 4 weeks after dosing

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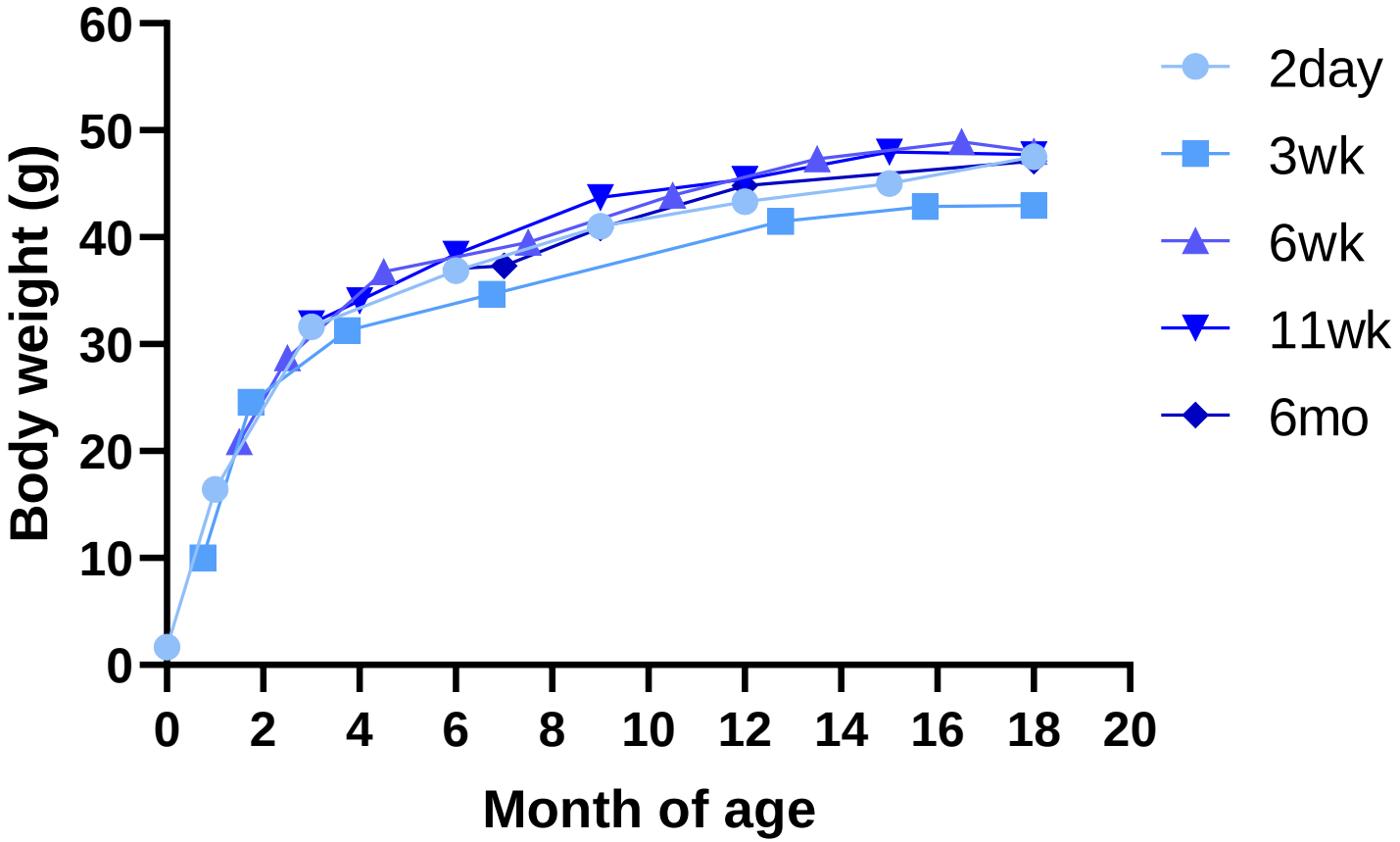
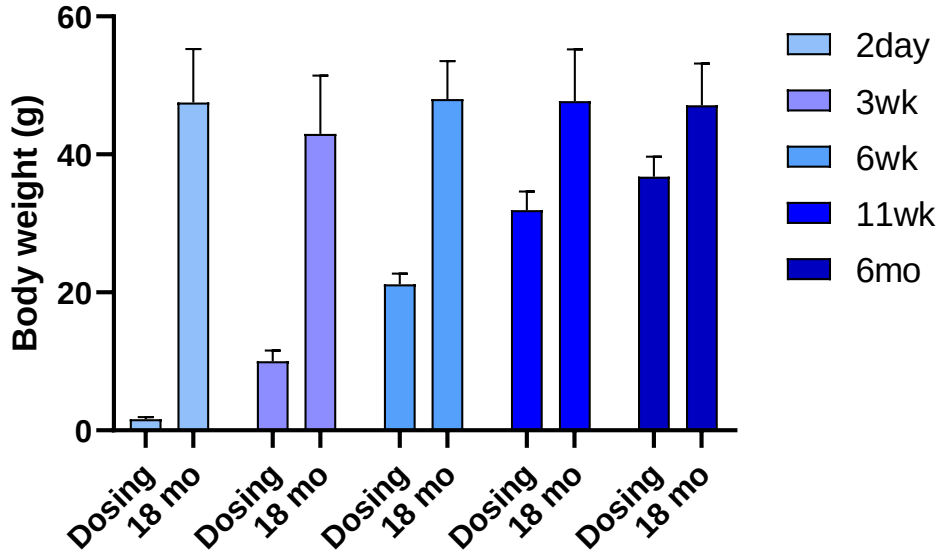
Significant growth in young animals in first months of life





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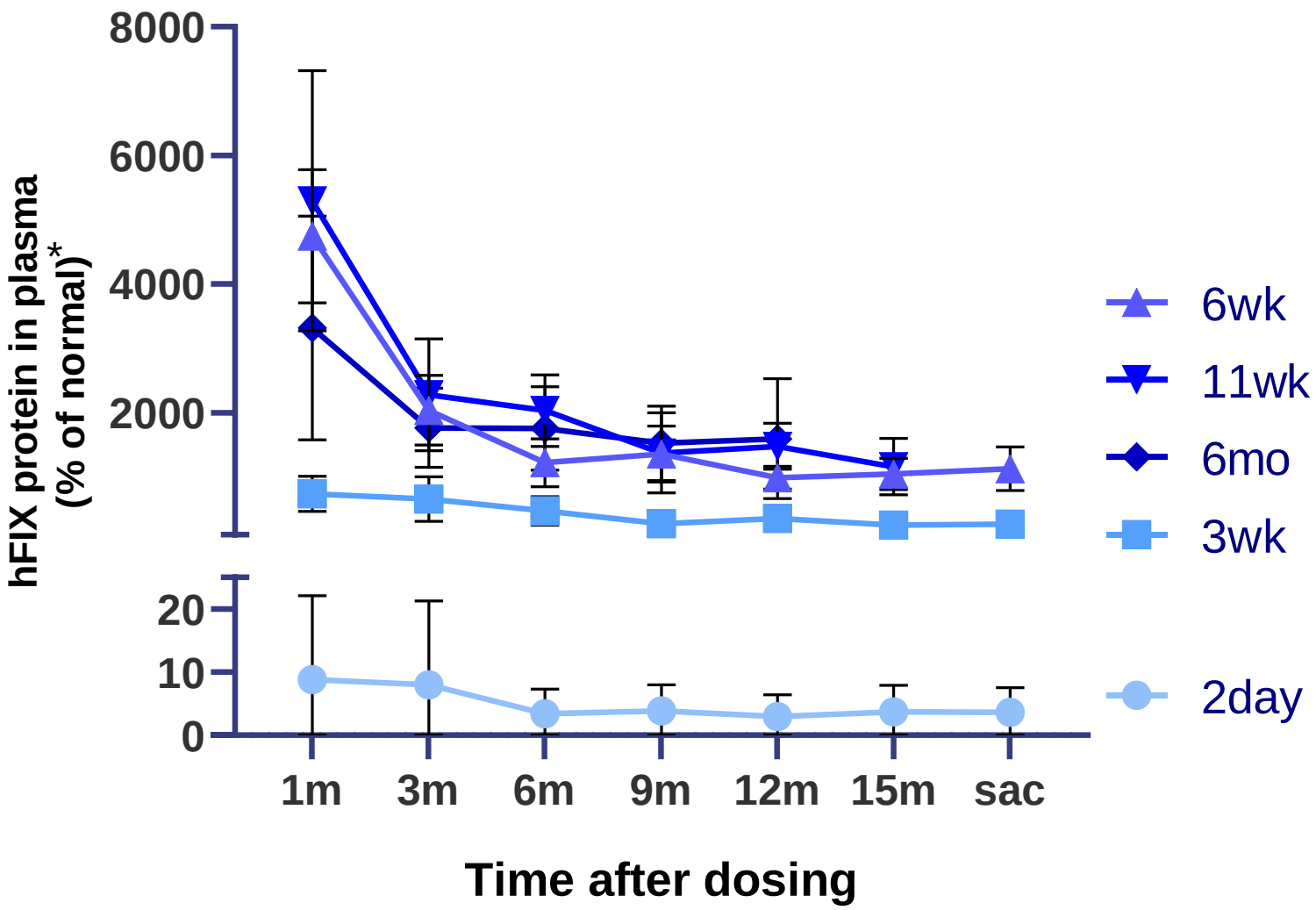
Sustained circulating hFIX protein levels up to 1.5 years



Group	Mean hFIX% @18 mo	Mean total gc/animal injected
2 day	3.7	$4.0x10^{11}$
3 wk	269	$2.3x10^{12}$
6 wk	1129	$4.8x10^{12}$
11 wk	1161	$7.4x10^{12}$
6 mo	1593	$8.5x10^{12}$

*measured against normal human pooled plasma calibrator

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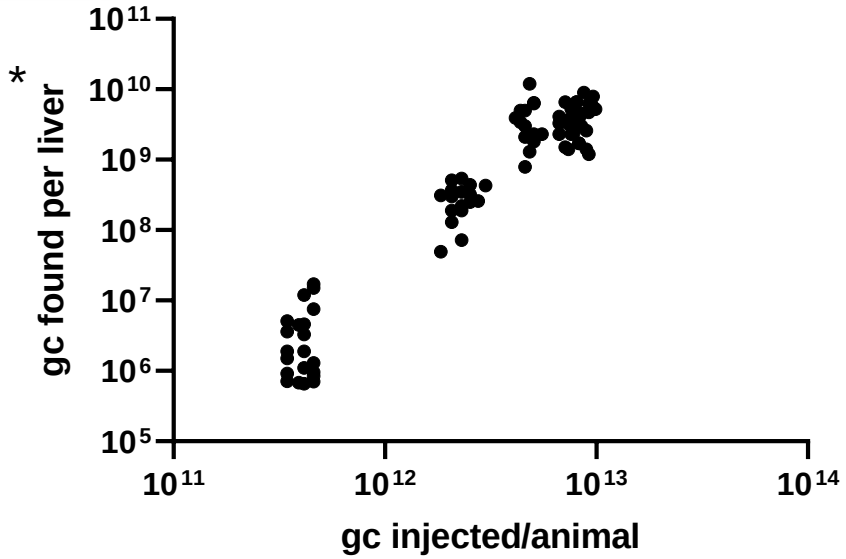
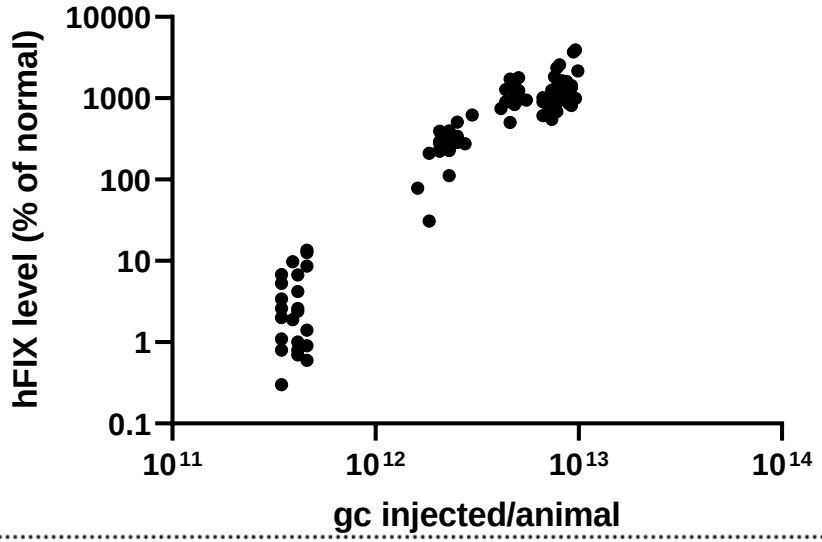
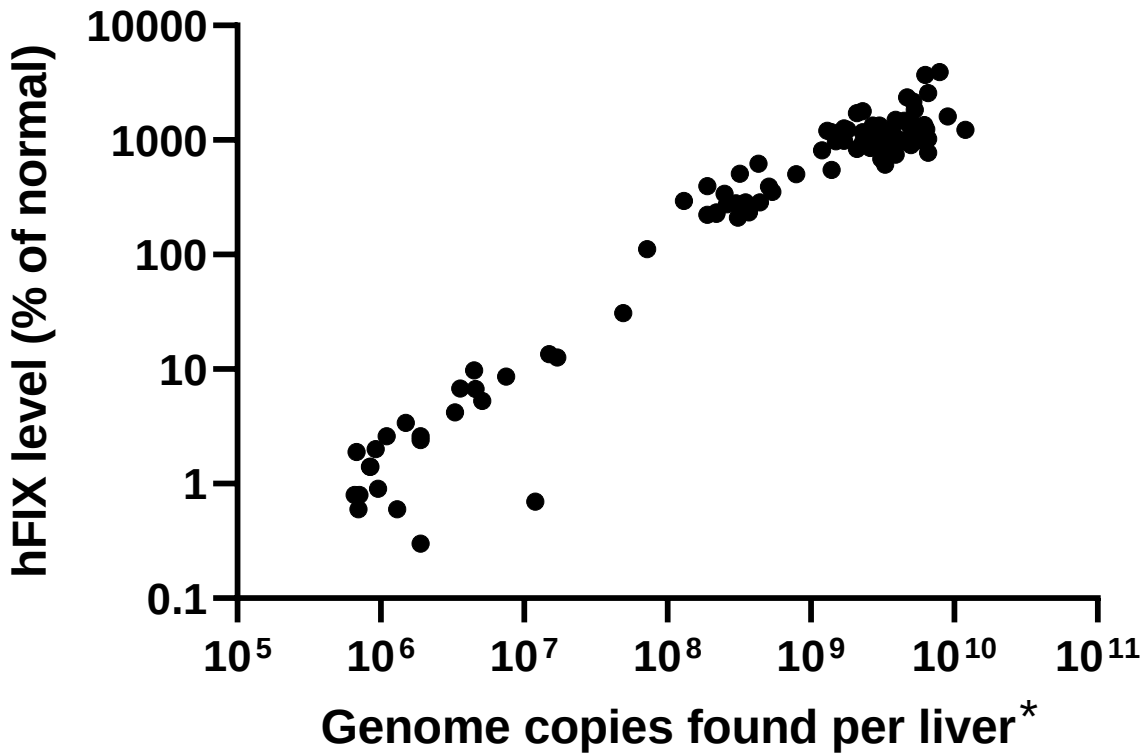
Strong correlation between genome copies injected, liver hFIX vector DNA and circulating hFIX protein at 1.5 years







*genome copies were detected per µg of genomic DNA
Assumptions: 1 µg of gDNA is approx 1^{e}5 cells and 1 gram of murine liver is approx 1^{e}8 cells. liver weight at necropsy for calculations

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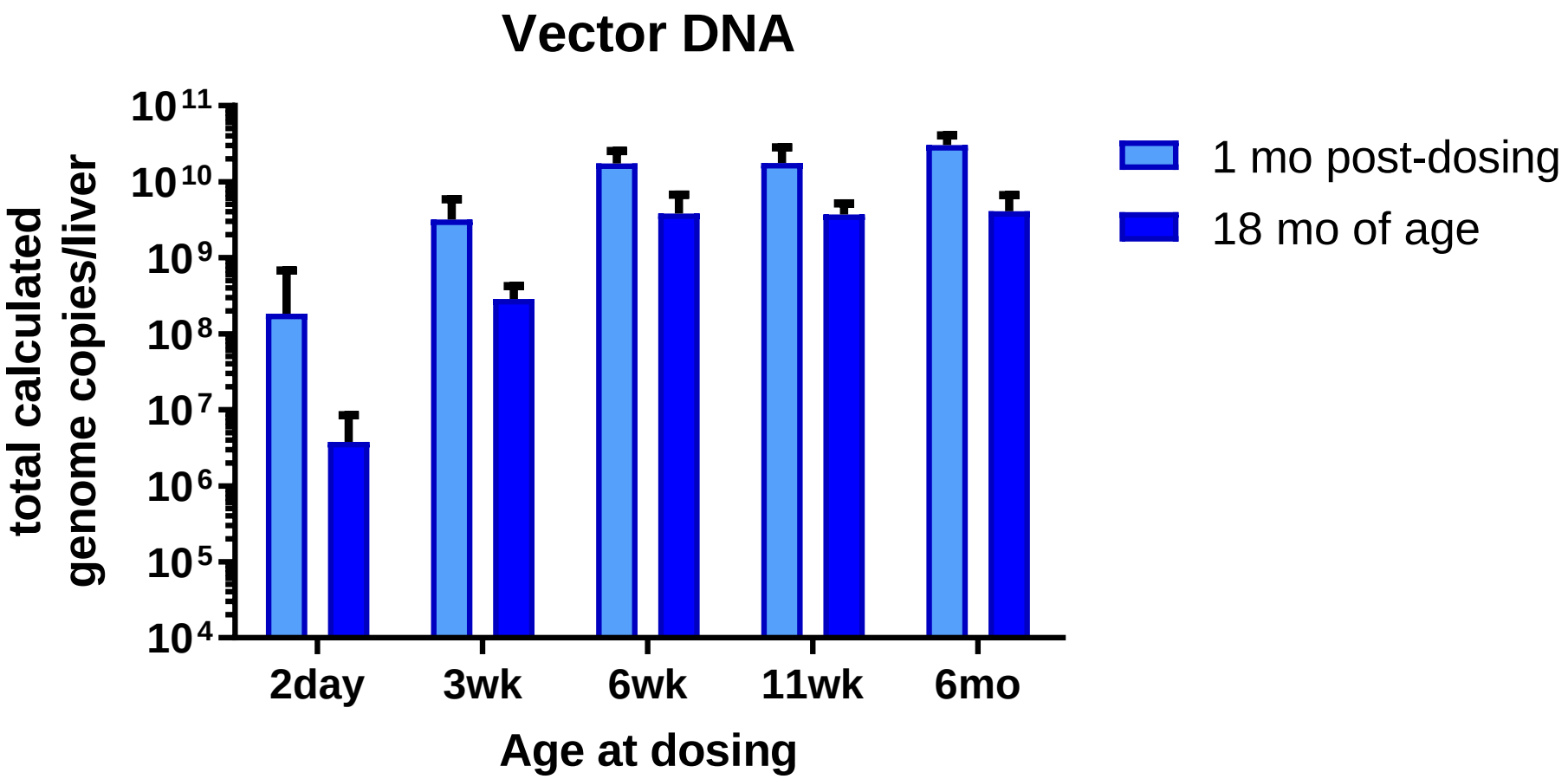
Sustained vector DNA in the liver up to 1.5 years



Sustained expression is not caused by integration

Vector DNA sequences in the liver were present almost exclusively as non-integrated **episomal forms**

- LAM-PCR[6] and integration site analysis on livers collected 6 months after dosing
- Animals treated with $2.3x10^{14}$ gc/kg AAV5-hFIX at 6 weeks of age

Neonatal administration of AAV leads to a life-long expression that was mediated by transcriptionally active double-stranded episomes and not integrated vector DNA[7]

6 Schmidt et al 2007
7 Bortolussi et al 2014

AAV administration leads to sustained transgene expression

AAV5-hFIX treatment of juvenile mice leads to life-long expression of hFIX

- The total number of genomic transgene copies was remarkably constant during the life of the mice
- hFIX protein level was stable and sustained until 18 months of age

Life-long expression is not due to integration of the transgene DNA

- AAV vector has been shown to establish stable replicating episomes in in vitro transgene-expressing colonies[8]
- Our data and similar studies[7] suggest that AAV concatemers are preserved long-term even in organs with a relatively high cell turnover

7 Bortolussi et al 2014
8 Hagedorn et al 2017

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