

Supplemental information

**Preclinical development of TAK-754,
a high-performance AAV8-based vector
expressing coagulation factor VIII**

Johannes Lengler, Markus Weiller, Franziska Horling, Josef Mayrhofer, Maria Schuster, Falko G. Falkner, Irene Gil-Farina, Matthias Klugmann, Friedrich Scheiflinger, Werner Hoellriegl, and Hanspeter Rottensteiner

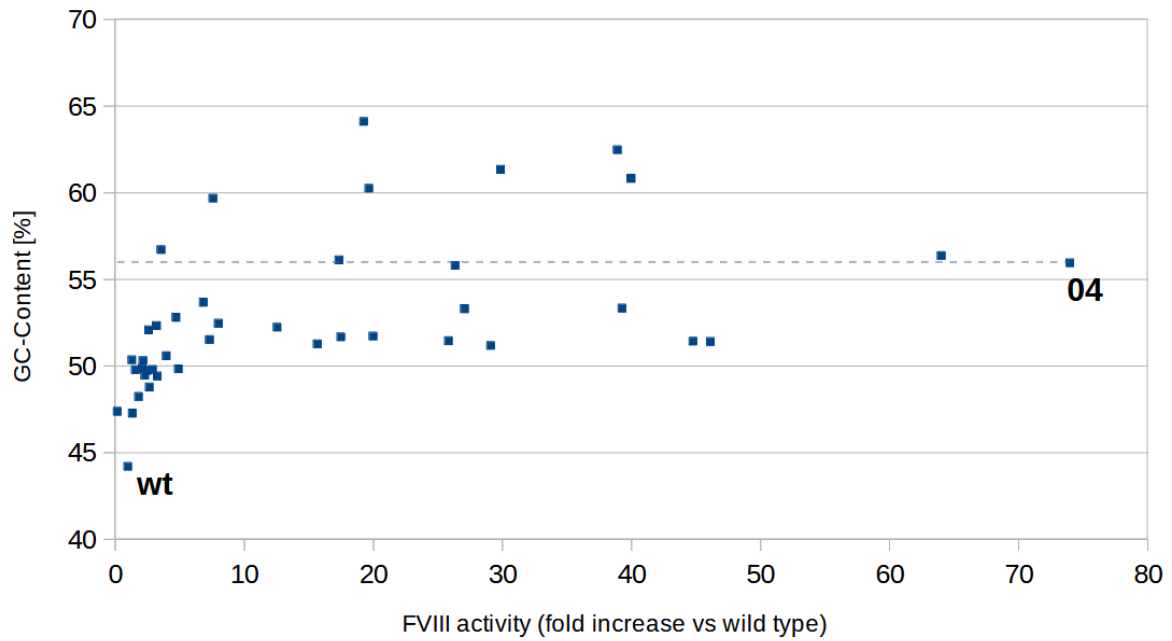


Figure S1. Correlation between GC content (%) of the screened *F8* nucleotide sequences and FVIII activity of the corresponding AAV vectors *in vivo*. The dashed line indicates the GC content of the native AAV8 capsid nucleotide sequence. The wild-type (wt) sequence, which had the lowest GC content (44.19%) of all constructs tested, and the Seq04 (04) lead candidate sequence, whose GC content (55.94%) matches that of AAV8, are highlighted. Sequences with a GC content below 50% resulted in low FVIII activity levels. See Supplemental Table 1 for actual FVIII activity and GC content numbers of all screened sequences.

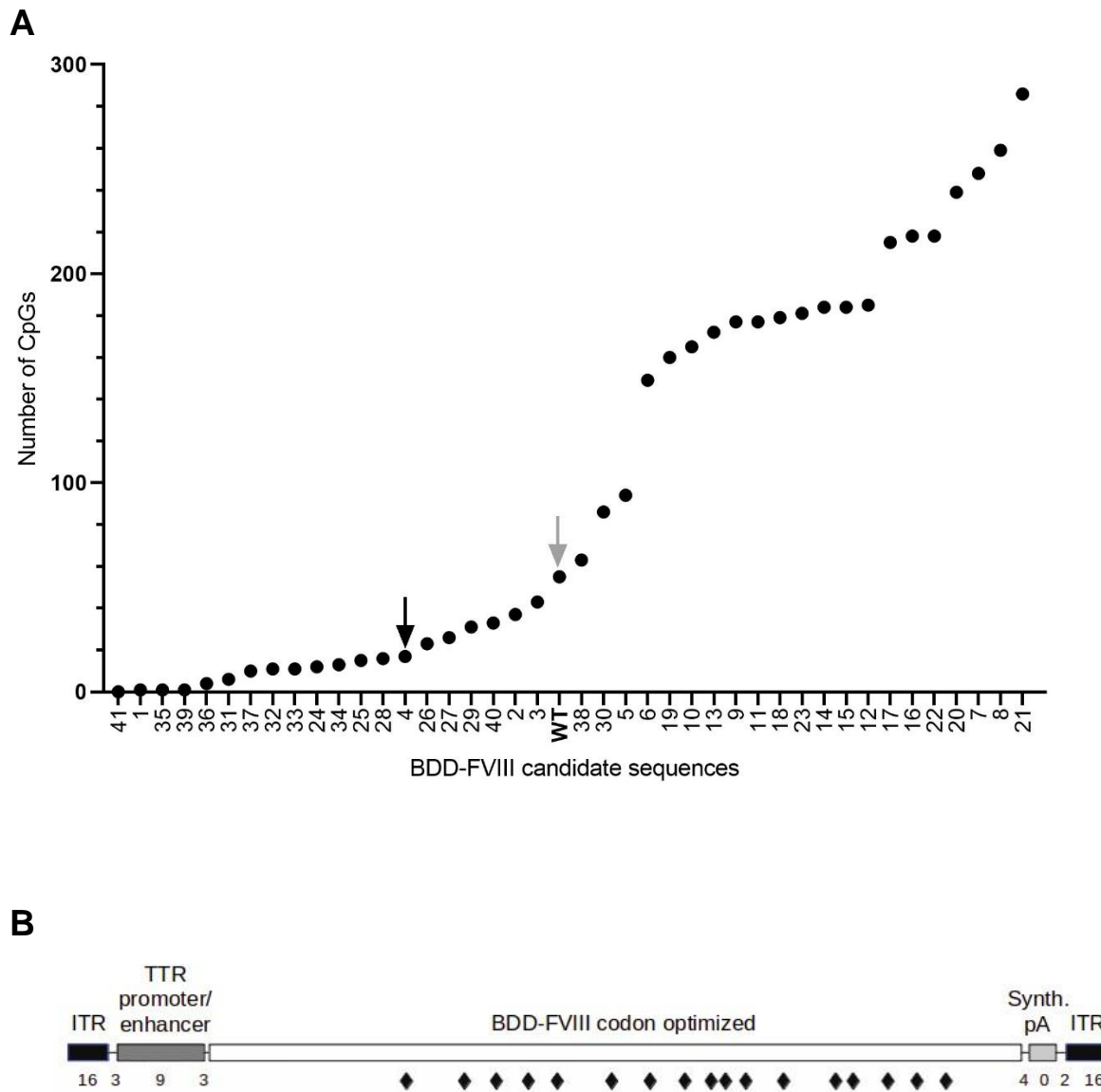


Figure S2. (A) CpG content of the screened *F8* nucleotide sequences. Sequences were sorted by their number of CpG motifs. The number of CpG motifs present in the Seq04 lead candidate (17, indicated by a black arrow) is lower compared to that of the wild-type (wt) nucleotide sequence (52, indicated by a gray arrow). (B) Distribution of CpG motifs in the TAK-754 expression cassette. The 17 CpG motifs of Seq04 are dispersed over the length of the BDD-*F8* transgene. Additional CpG motifs are present in the ITR and promoter regions as well as in the restriction sites used for cloning the expression cassette.

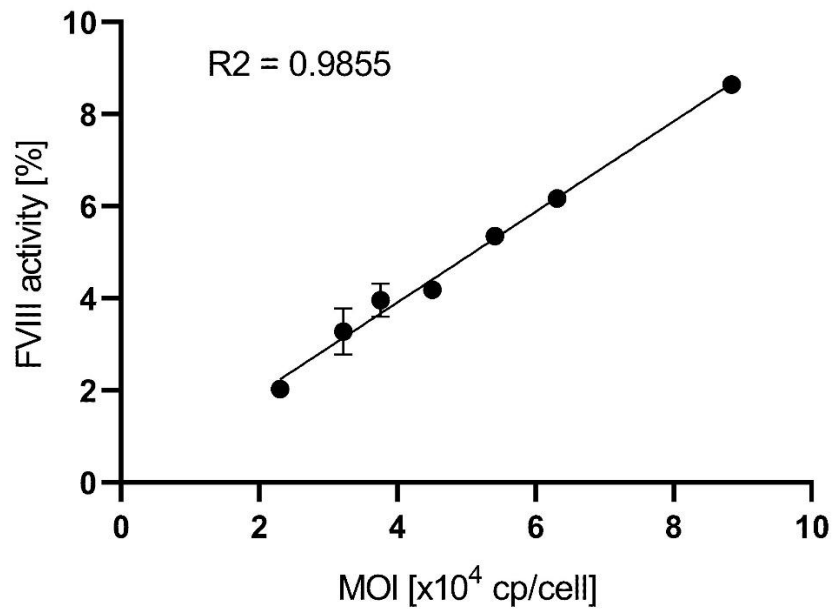


Figure S3. In vitro biopotency assay. HepG2 cells were transduced with increasing multiplicities of infection (MOI) of the TAK-754 vector and FVIII activity was measured in the cell culture media after 3 days. A dose-dependent increase in FVIII activity levels was observed. Data shown represent the mean \pm standard deviation (SD).

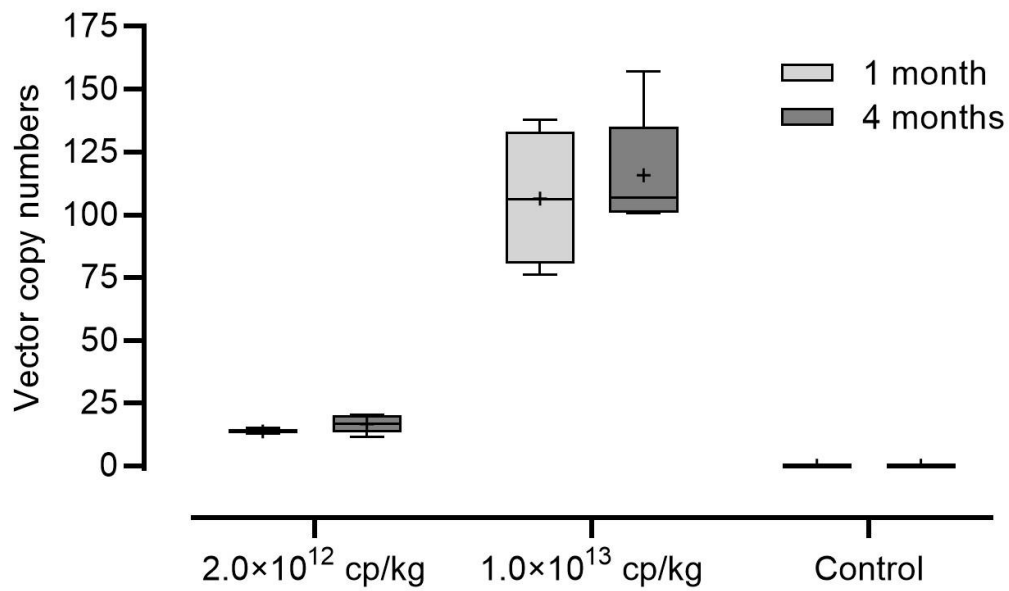


Figure S4. Vector copy numbers in the livers of FVIII ko mice at 1 month or 4 months after injection of 2.0×10^{12} CP/kg and 1.0×10^{13} CP/kg TAK-754. Data are shown as box-and-whiskers plots, with the median indicated by a horizontal line and the mean represented by a cross.

Table S1. GC content of the screened *F8* nucleotide sequences and FVIII activity of the corresponding AAV vectors *in vivo*.

seq ID ^a	GC-Content [%]	FVIII activity [relative to wt]
35	47.37	0.19
Wt	44.19	1.00
12	50.34	1.31
39	47.26	1.35
13	49.77	1.58
19	48.22	1.84
15	49.84	2.12
14	50.30	2.18
06	49.45	2.32
11	49.73	2.50
25	52.06	2.63
18	48.77	2.67
10	49.79	2.91
24	52.31	3.22
02	49.40	3.30
08	56.70	3.58
05	50.57	3.98
28	52.79	4.74
09	49.82	4.93
17	53.66	6.86
31	51.51	7.33
20	59.67	7.60
03	52.45	8.02
40	52.22	12.57
34	51.26	15.69
26	56.10	17.37
01	51.67	17.51
21	64.10	19.29
07	60.24	19.68
36	51.71	20.00
37	51.44	25.85
27	55.78	26.37
29	53.29	27.09
41	51.17	29.12
23	61.32	29.90
22	62.46	38.93
38	53.32	39.29
16	60.81	40.00
32	51.42	44.80
33	51.39	46.15
30	56.36	64.03
04	55.94	74.00

^a Sequences sorted according to increasing FVIII activities.

Table S2. Sequencing results and Integration sites (IS) retrieval

Dose [cp/kg]	Time [months]	Animal	Raw Reads	Potential IS Reads	Used Reads	Unique IS Mappable	Frequency of IS [%]^a
2.0×10 ¹²	1	1	1062425	19279	162	8	0.0018
		2	714172	15303	114	5	0.0011
		3	1066211	19461	272	13	0.0029
		4	1186995	16961	297	26	0.0058
2.0×10 ¹²	4	5	815345	4150	481	17	0.0038
		6	665375	5076	3024	5	0.0011
		7	729566	22584	1641	16	0.0036
		8	1020653	14802	844	19	0.0042
		9	717888	9667	965	18	0.0040
1.0×10 ¹³	1	10	871515	19345	1167	54	0.0120
		11	921756	20918	618	53	0.0118
		12	1034457	9528	909	30	0.0067
		13	1126924	26541	744	38	0.0084
1.0×10 ¹³	4	14	899376	2835	1142	7	0.0016
		15	890956	4400	360	21	0.0047
		16	809562	4603	413	21	0.0047
		17	826638	6998	1033	42	0.0093
		18	815572	4955	618	21	0.0047
Buffer	1	98	46	0	0	0	0.0000
	4	99	1336	4	0	0	0.0000

^a IS Frequency = number of mappable IS / number of analyzed cells (=450,000 per 3 µg DNA)