OMTM, Volume *9*

Supplemental Information

A Rationally Engineered Capsid Variant of AAV9 for Systemic CNS-Directed and Peripheral Tissue-Detargeted Gene Delivery in Neonates

Dan Wang, Shaoyong Li, Dominic J. Gessler, Jun Xie, Li Zhong, Jia Li, Karen Tran, Kim Van Vliet, Lingzhi Ren, Qin Su, Ran He, Jason E. Goetzmann, Terence R. Flotte, Mavis Agbandje-McKenna, and Guangping Gao

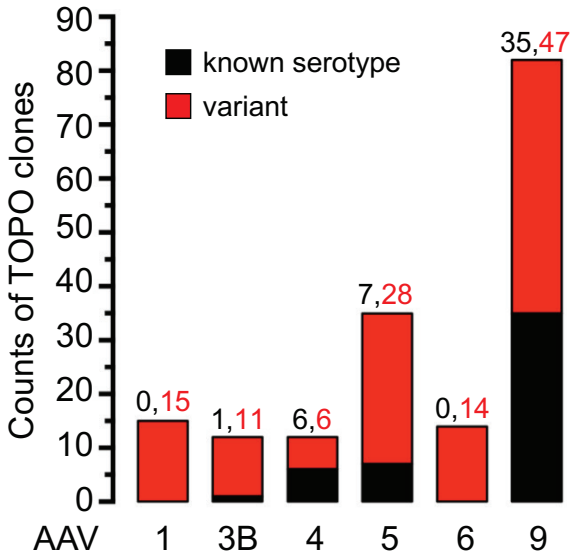

Figure S1. Stacked histogram showing the number of AAV capsid sequences isolated from a chimpanzee. The counts of TOPO clones representing known AAV serotypes 1, 3B, 4, 5, 6, and 9 are shown in black, and the counts of variants are shown in red. The exact counts of known serotypes and variants are labeled above each bar.

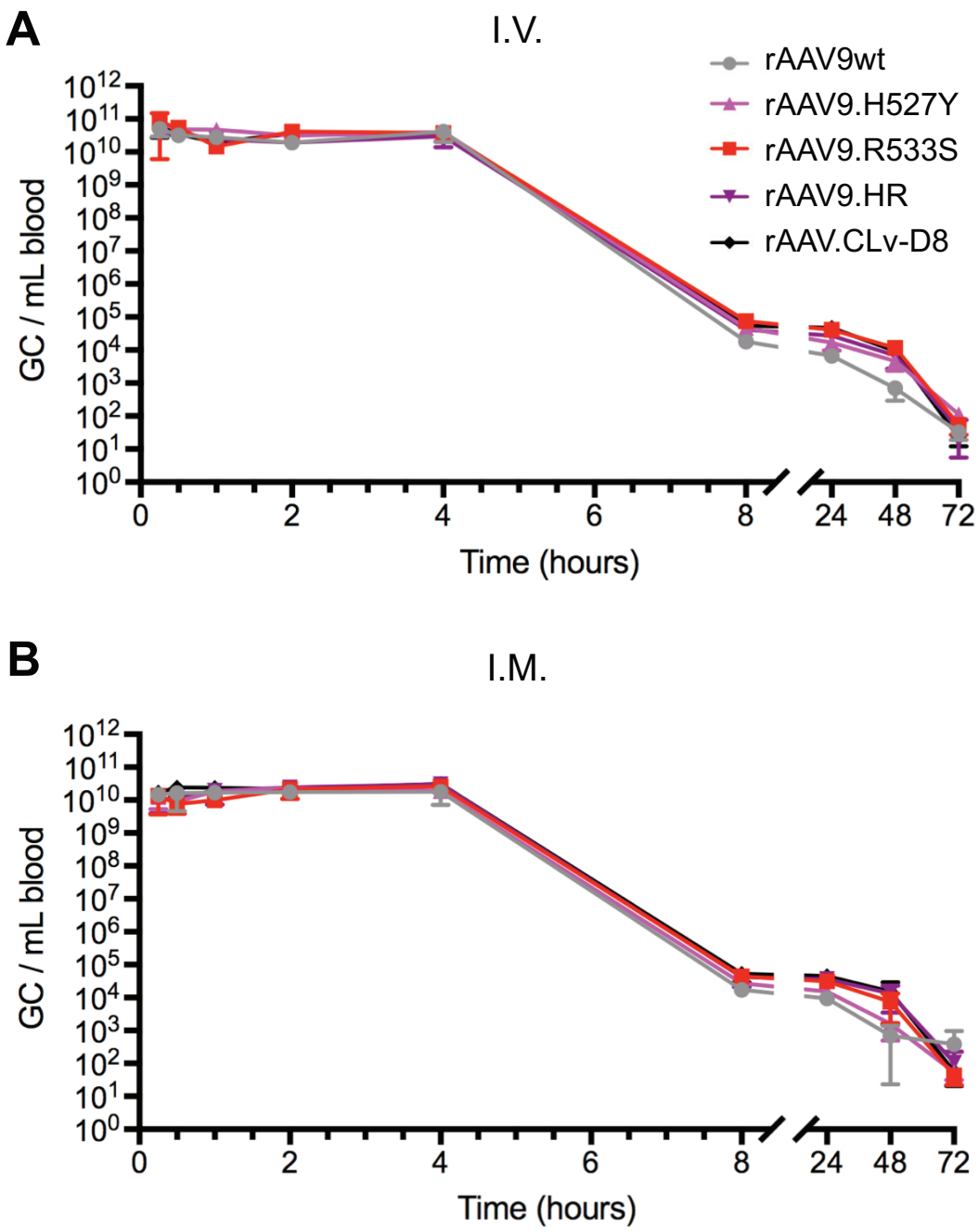

Figure S2. Blood clearance of vectors following delivery into mice. The same single-stranded recombinant AAV genome expressing luciferase was packaged into each of 5 capsids as indicated. Following I.V. (**A**) or I.M. (**B**) injection of 1.0E+11 GC rAAV into 6-week old male mice, the rAAV genome copies per milliliter of blood (GC/mL blood) were plotted over time after vector delivery.

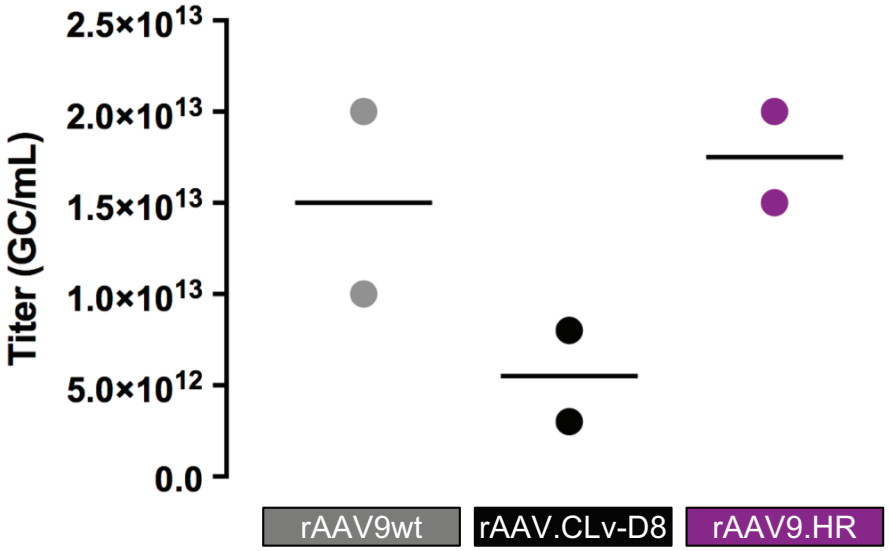

Figure S3. Packaging efficiency of the rAAV9wt, rAAV.CLv-D8, and rAAV9.HR vectors harboring the same single-stranded luciferase-expressing genome. Two preparations with each capsid were included in the comparison.

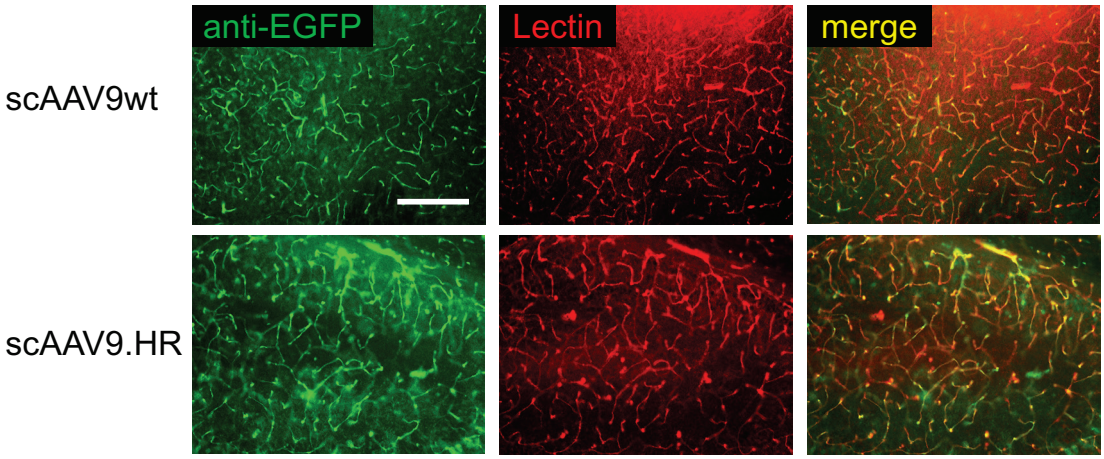

Figure S4. Blood vessel transduction in adult mice following I.V. delivery of 4E+12 GC of scAAV9wt-EGFP or scAAV9.HR-EGFP. Immunofluorescence staining images of brain sections showing the co-localization of transduction signal (anti-EGFP) and vasculature (Lectin staining). 6-week-old mice were treated with EGFP-expressing vectors as described in Figure 3. Scale bar: 200 microns.

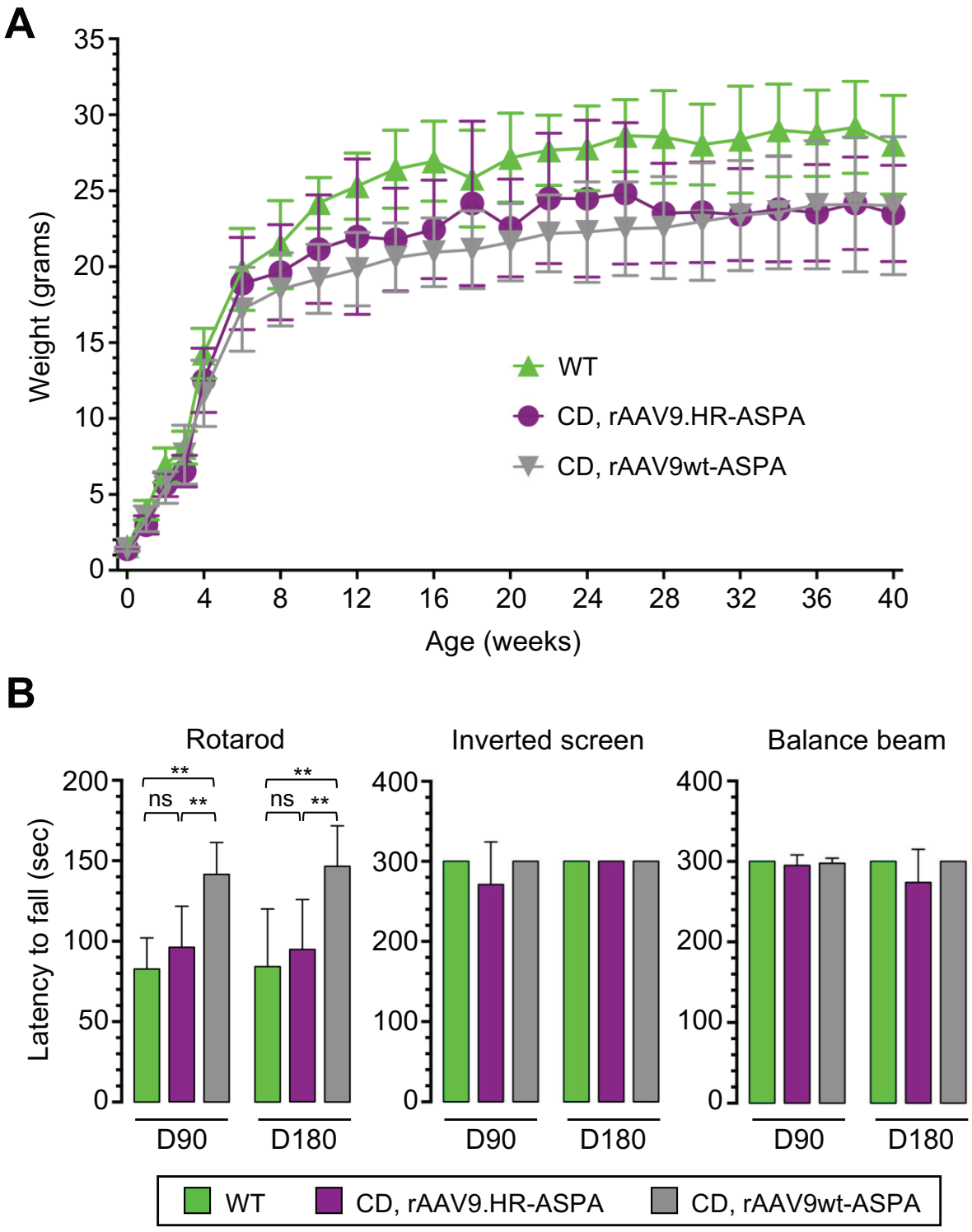

Figure S5. I.V. delivery of rAAV9.HR-ASPA and rAAV9wt-ASPA at P1 to CD mice rescued body weight loss and behavioral deficits over an extended period of time. **A.** Body weight curves plotted against age of untreated WT mice (green), untreated CD mice (red) and CD mice treated with rAAV9.HR-ASPA (purple) or rAAV9wt-ASPA (gray). N=10 mice per group. **B.** Motor function performance of untreated WT mice (green), untreated CD mice (red) and CD mice treated with rAAV9.HR-ASPA (purple) or rAAV9wt-ASPA (gray) in three behavioral tests including rotarod test, inverted screen test and balance beam test. Tests were performed when mice reached 90 days old (D90) or 180 days old (D180). N=6 mice per group per test. One-way ANOVA was performed ($p < 0.01$ for rotarod test; not significant for the other tests). $^{**}p < 0.01$ and ns: not significant by Tukey's multiple comparisons test. All data are presented as mean and standard deviation.

Table S1. Amino acid residue changes of the AAV9 variants isolated from chimpanzee tissues.

Tissue	Template Source	ID	# of residue changes	VP1u* (aa 1-137)	VP1/2* (aa 138-202)	VP3* (aa 203-736)
Brain	RNA	CBr-E3	1	N66S		
		CBr-E4	1			M471T
		CBr-E1	2			S414G, N696D
		CBr-E2	2			H292R, T652A
		CBr-e5	2			K316R, N598S
		CBr-E7	2	A121D		N305del
		CBr-E8	2			V239A, I560T
		CBr-E5	3			N409S, V572A, Y701H
		CBr-E6	4			G282R, N305del, V344A, K666E
Liver	DNA	CLv1-8	6			H255R, L317P, Y445H, H527Y, R533S, I647T
		CLv1-2	7	K123R		V322I, S348L, Y445H, H527Y, R533S, I647T
		CLv1-1	8	G49D, A70V		R238G, Y445H, S458P, H527Y, R533S, I647T
		CLv1-7	8			I240V, Y426H, Y445H, P468H, H527Y, R533S, I647T, E684G
		CLv1-3	9	N66S	P183S	P367S, Y445H, H527Y, R533S, N552D, M559I, I647T
		CLv1-4	9	D97G		D219G, I240V, E361G, Y445H, N477S, H527Y, R533S, I647T
	RNA	CLv-D6	1			Y445H
		CLv-R3	1			W298R
		CLv-R8	1			S708P
		CLv-D3	2	L131P		V580A
		CLv-D4	2	L131P		Q299R
		CLv-D5	2	P89L		Y445H
		CLv-R2	2			L439P, S722G
		CLv-R4	2			D231N, S469G
		CLv-D1	3	A121D	D154G	Y445H
		CLv-R5	3	K51R		I334V, E723G

		CLv-D7	4			N263T, Q442X, Y445H, G604D
		CLv-D8	**4**			**Y445H, H527Y, R533S, I647T**
		CLv-R1	4	K33R		Y350C, M436T, Y577H
		CLv-D2	5	L7F	P191S	F400L, Y445H, M524V
		CLv-R9	6	D76N, Y93C	L125P, G199R	F275L, A523T
		CLv-R7	8	H38R, D87G, Y90C	K161R	T333A, N452D, N519D, D626G
Lung	RNA	CLg-F5	2	Y93C		F275L
		CLg-F2	4	D76N, Y93C	G199R	F275L
		CLg-F7	4	Y93C		F275L, G276D, D609G
		CLg-F3	5	Y93C		F275L, K316E, F413S, V489A
		CLg-F1	6	E74V, Y93C		F275L, M373T, T729P, L736F
		CLg-F4	6	Y93C	S195P	F275L, N452S, N498S, P603S
Spleen	DNA	CSp-10	1			M640V
		CSp-3	1			F275L
		CSp-9	2			N668D, E698V
		CSp-11	3			I302T, F400I, M640V
		CSp-2	3			F543S, D556N, G639E
		CSp-4	3		S181P	D370G, V493A
		CSp-6	3	E99G	D184V	T548A
		CSp-1	4	L7P, Y90H		M640V, K666R
		CSp-7	4	W22R, L126X		L432P, M640V
		CSp-8	6			M203I, Q259R, Q321R, A335T, Q495R, M640V

*VP1u: VP1 unique region; VP1/2: Shared region between VP1 and VP2; VP3: VP3 region.