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Supplemental Information

Novel MECP2 gene therapy is effective in a multicenter study using two mouse models of Rett syndrome and is safe in non-human primates

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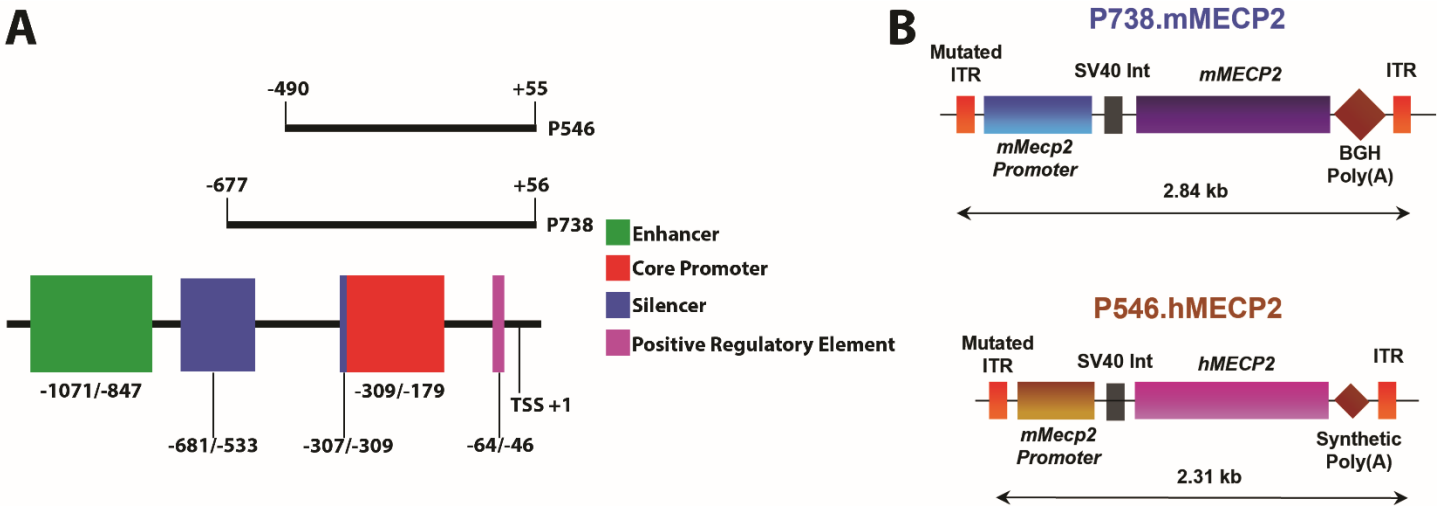

Fig. S1: Schematic of the P546 and P738 Promoters and Vector Constructs. (A) Schematic shows representative drawing of the P546 and P738 promoters relative to the annotated *Mecp2* mouse promoter region. (B) Representative drawing of the P738.mMECP2 vector from Garg *et al*, 2013 and the P546.hMECP2 vector used in the current study.

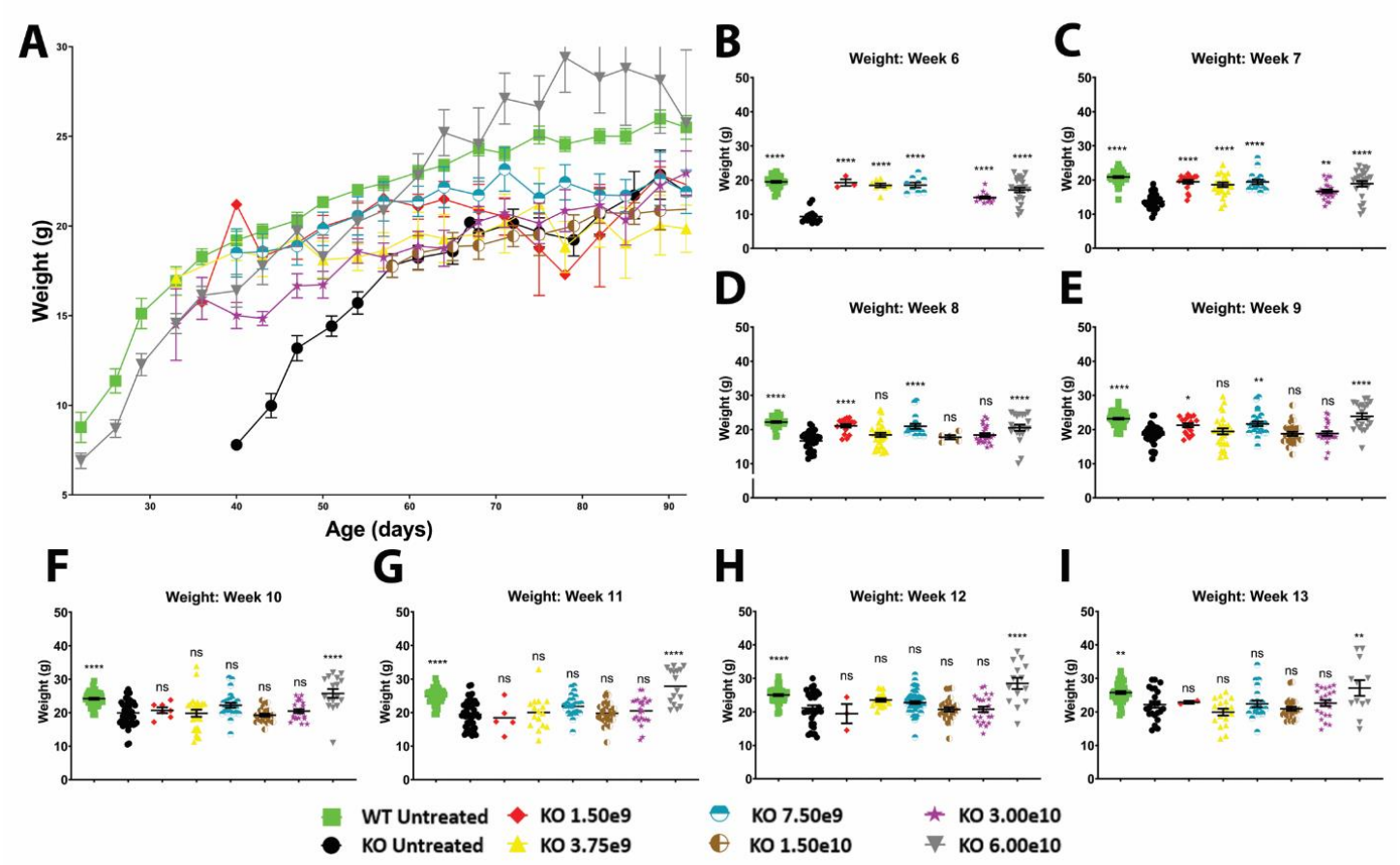

Fig. S2: scAAV9.P546.MECP2 Treatment Improves KO Weight During Early Disease Course.

(A) Weight is plotted for untreated WT, untreated KO and vector treated KO mice with a table showing the decreasing difference in weight between untreated WT and KO mice between week 6-13. (B-I) Average weight per treatment group is shown in for week 6-13. (B) Week 6: all vector treated KO groups have significantly higher weight than untreated KO mice ($p<0.0001$). (C) Week 7: All vector treated KO groups have significantly higher weight than untreated KO mice ($p<0.0001$ for all except 3.00×10^{10}, $p=0.0036$). (D) Week 8: half of the vector treated KO groups have significantly higher weight than untreated KO mice (untreated WT, $p<0.0001$; 1.50×10^{9}, 7.50×10^{9}, 6.00×10^{10}, $p<0.0001$; KO 3.75×10^{9}, $p=0.2121$; KO 1.5×10^{10}, $p=0.9998$; KO 3.0×10^{10}, $p=0.3615$). (E) Week 9: half of the vector treated KO groups have significantly higher weight than untreated KO mice (untreated WT, $p<0.0001$; 1.50×10^{9}, $p=0.0282$; 3.75×10^{9}, $p=0.9745$; 7.50×10^{9}, $p=0.0008$; 1.5×10^{10}, $p>0.9999$; 3.00×10^{10}, $p>0.9999$; 6.00×10^{10}, $p<0.0001$). (F) Week 10: KO mice treated with the highest dose of vector had significantly higher weight than untreated KO mice (untreated WT, $p<0.0001$; 1.50×10^{9}, $p>0.9999$; 3.75×10^{9}, $p>0.9999$; 7.50×10^{9}, $p=0.1470$; 1.5×10^{10}, $p>0.9999$; 3.00×10^{10}, $p>0.9999$; 6.00×10^{10}, $p<0.0001$). (G) Week 11: KO mice treated with the highest dose of vector had significantly higher weight than untreated KO mice (untreated WT, $p<0.0001$; 1.50×10^{9}, $p>0.9999$; 3.75×10^{9}, $p>0.9999$; 7.50×10^{9}, $p=0.0951$; 1.5×10^{10},

p>0.9999; 3.00 x 10^{10}, p=0.9866; 6.00 x 10^{10}, p<0.0001). (H) Week 12: KO mice treated with the highest dose of vector had significantly higher weight than untreated KO mice (untreated WT, p<0.0001; 1.50 x 10^{9}, p>0.9999; 3.75 x 10^{9}, p>0.4738; 7.50 x 10^{9}, p=0.5593; 1.5 x 10^{10}, p>0.9999; 3.00 x 10^{10}, p>9999; 6.00 x 10^{10}, p=0.0068). (I) Week 13: KO mice treated with the highest dose of vector had significantly higher weight than untreated KO mice (untreated WT, p=0.0050; 1.50 x 10^{9}, p>0.9999; 3.75 x 10^{9}, p>0.7524; 7.50 x 10^{9}, p>0.9999; 1.5 x 10^{10}, p>0.9918; 3.00 x 10^{10}, p>9999; 6.00 x 10^{10}, p=0.0068). Statistics applied show the mean of each group compared with the mean of untreated KO via ANOVA with Tukey's honest significance post hoc test. Weights of 1.5 x 10^{10}vg were not recorded prior to week 8.

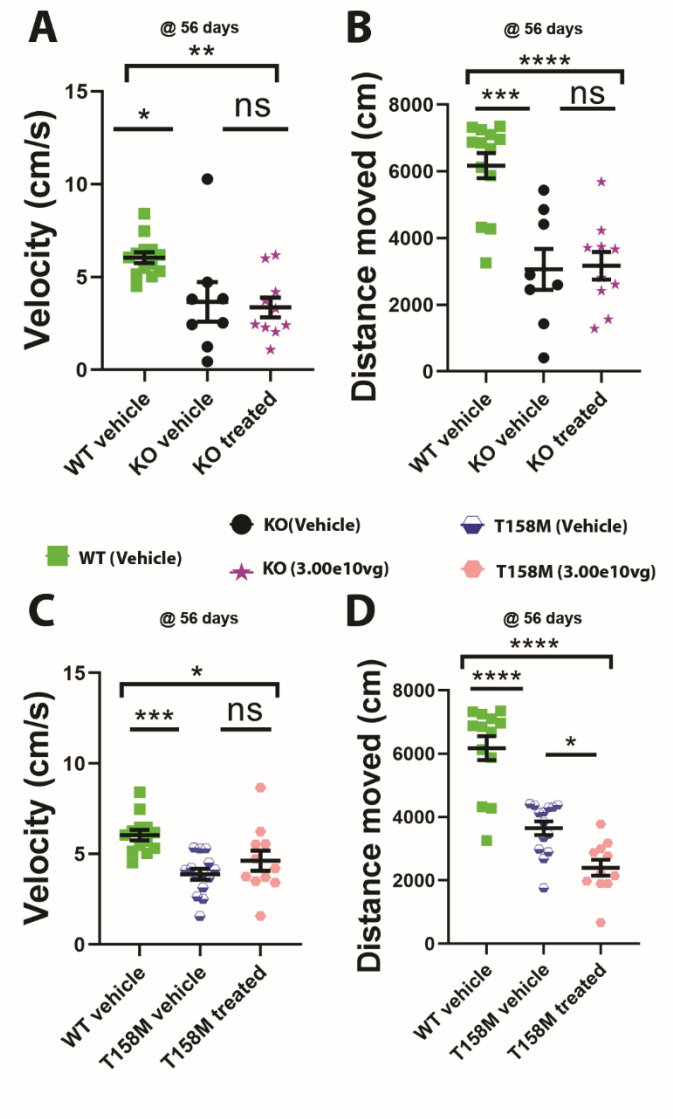

Fig. S3: ICV Delivery of SCAAV9.P546.*MECP2* Does not Improve Open Field Measures in KO and T158M Mice at 56 Days. Open field performance is not ameliorated in vector treated KO and T158M mice at 56 days. (A) Vehicle treated WT mice had a significantly higher velocity than vehicle treated KO mice or vector treated KO mice (vehicle treated KO, p=0.0261; vector treated KO, p=0.0068) and there was no difference between vehicle treated KO mice and vector treated KO mice, p=0.9428. (B) Vehicle treated WT mice had a significantly higher distance than vehicle treated KO mice or vector treated KO mice (vehicle treated KO, p=0.0001; vector treated KO, p<0.0001) and there was no difference between vehicle treated KO and vector treated KO, p=0.9876. (C) Vehicle treated WT mice had a significantly higher velocity than vehicle treated T158M mice or vector treated T158M mice (vehicle treated T158M mice, p=0.0006; vector treated T158M mice, p=0.0342) and there was no difference between vehicle treated T158M and vector treated T158M mice, p=0.3613. (D) Vehicle treated WT mice had a significantly higher distance than vehicle treated T158M mice and vector treated T158M mice, p<0.0001 for both comparisons, and there was a significant difference between vehicle treated T158M mice

and vector treated T158M mice, p=0.0141. Statistical significance was determined via ANOVA with Tukey's honest significance post hoc test. Values represent means ± SEM, * = p<0.05, ** = p<0.01, *** = p<0.001, **** = p<0.0001.

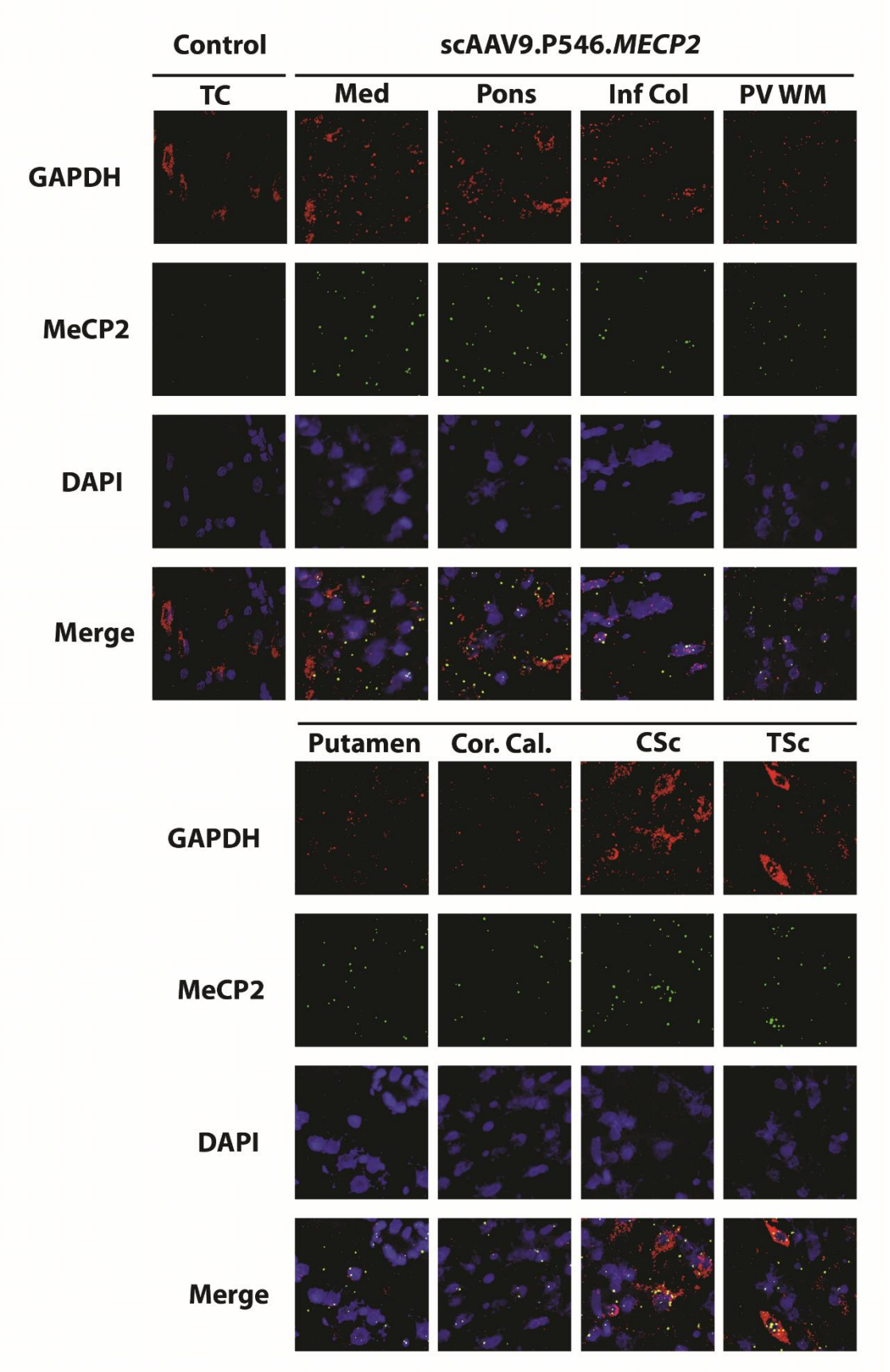

Fig S4. *In Situ* Hybridization Analysis in Brain Tissue from Control and SCAAV9.P546.*MECP2* Treated NHPs at 18 Months After a Single Intrathecal (IT) Dose of SCAAV9.P546.*MECP2* *In situ* hybridization analysis shows vector-

derived RNA transcripts in additional key brain regions from brains of SCAAV9.P546.*MECP2*-treated WT animals but not untreated controls. The figure shows probes against Gapdh (red) and vector-derived human *MECP2* mRNA (green) along with nuclear labeling (Dapi, blue). TC = Temporal Cortex; Med = Medulla; Inf. Col. = Inferior Colliculus; PV. WM. = Periventricular White Matter; Cor. Cal. = Corpus Callosum; CSc = Cervical Spinal Cord, TSc = Thoracic Spinal Cord.

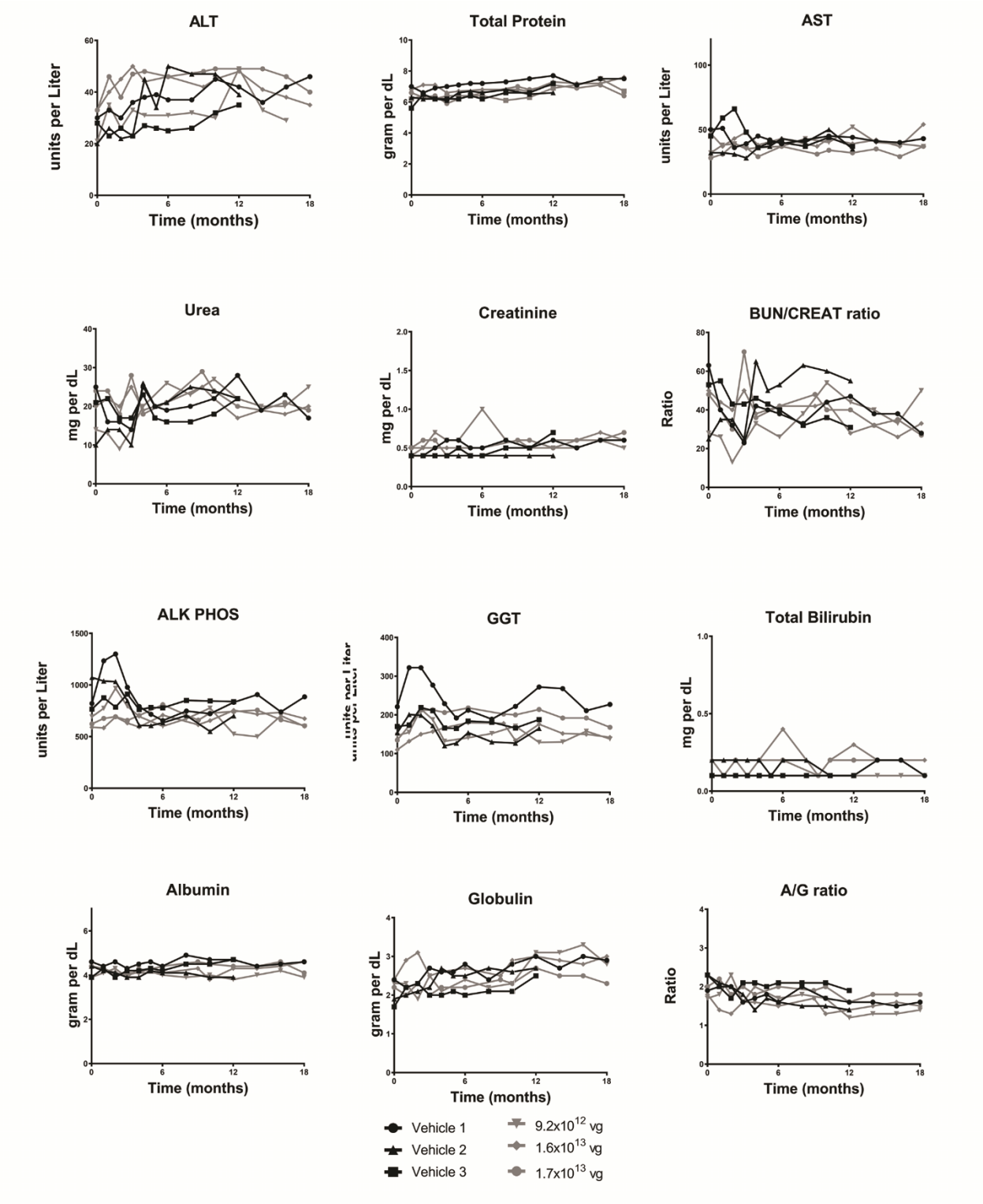

Fig. S5: Serum Chemistries Following Intrathecal Delivery of scAAV9.P546.*MECP2* in NHPS up to 18 Months Post Injection. Serum chemistry values are graphed over 18 months post-injection: alanine (ALT), total protein, aspartate (AST), urea, creatinine, blood urea nitrogen/creatinine ratio (BUN/CREAT ratio), alkaline phosphatase activity (ALK PHOS), gamma-glutamyl transferase (GGT), total bilirubin, albumin, globulin, and albumin/globulin ratio (A/G ratio).

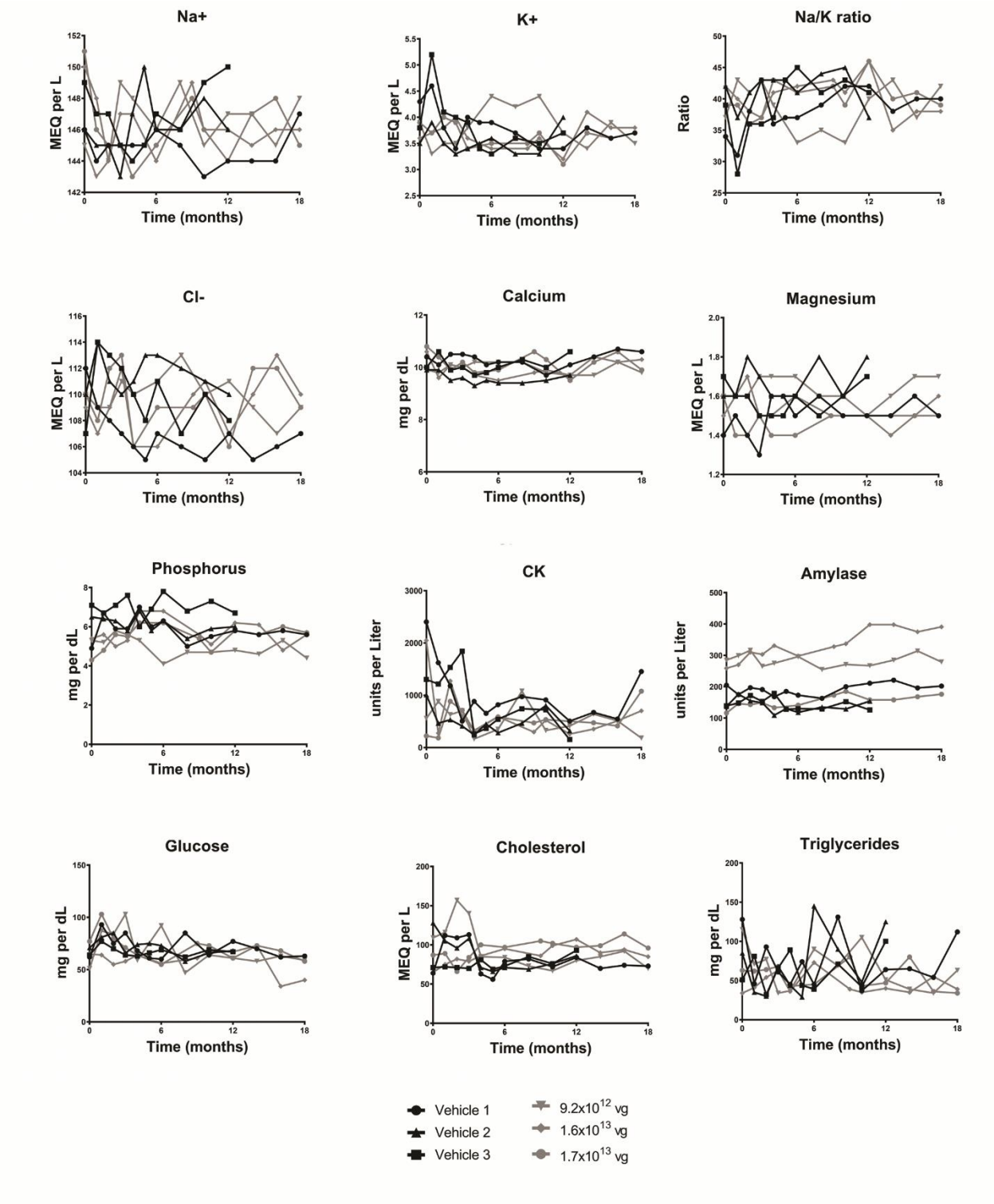

Fig. S6: Serum Chemistries following Intrathecal Delivery of scAAV9.P546.*MECP2* in NHPs up to 18 months Post Injection. Blood serum chemistry is graphed over 18 months post injection: sodium (Na+), potassium (K+), sodium/potassium ratio (Na/K ratio), chloride (Cl-), calcium, magnesium, phosphorus, creatine kinase (CK), amylase, glucose, cholesterol, and triglycerides.

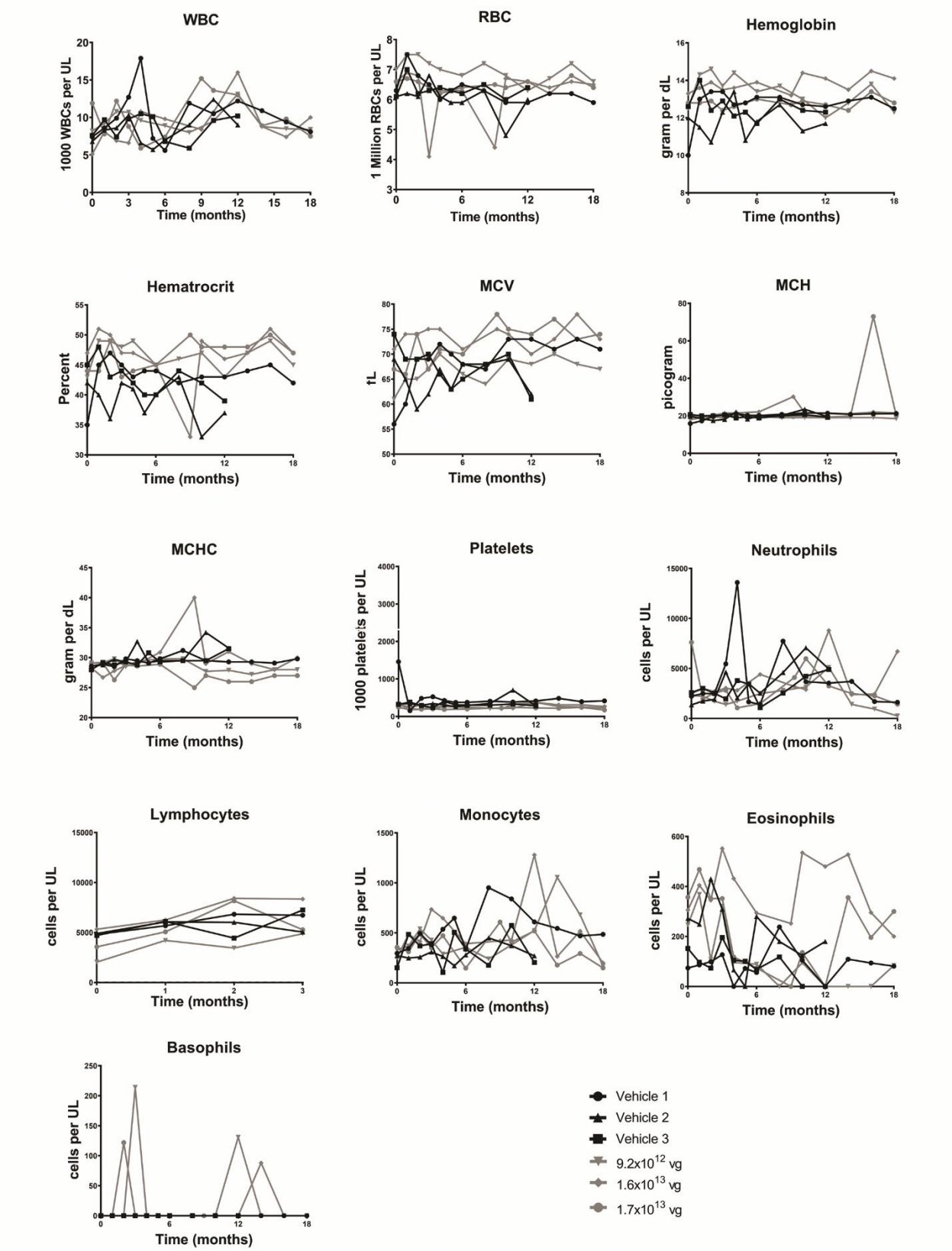

Fig. S7: Hematology Following Intrathecal Delivery of scAAV9.P546.*MECP2* in NHPS up to 18 months Post Injection. Hematology parameters are graphed over 18 months post injection: white blood cells (WBC), red blood cells (RBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean

corpuscular hemoglobin concentration (MCHC), platelets, neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

Table S1: Vector Titer

	Silver Staining Titer		ddPCR Titer	
scAAV9.P546.*MECP2* preparation	**Viral Titer (vg/mL)**	**Dose Range**	**Viral Titer (vg/mL)**	**Dose Range**
Mouse Preparation	$1.5x10^{13}$	$1.5x10^{9}$ vg – $6.00x10^{10}$ vg	$1.2x10^{13}$	$1.2x10^{9}$ vg – $4.00x10^{10}$ vg
NHP Preparation	$1.1x10^{13}$	$9.2x10^{12}$ vg – $1.7x10^{13}$ vg	$2.1x10^{13}$	$1.8x10^{13}$ vg – $3.2x10^{13}$ vg

Table S2: Aggregate Behavioral Score Untreated WT vs. Treated KO Comparison

Time point	Tukey's Honest Significance Test	Mean Diff.	95.00% CI of diff.	Significance	Adjusted P Value
60 Days	WT Untreated vs. KO Untreated	-3.228	-3.879 to -2.577	****	<0.0001
60 Days	WT Untreated vs. KO 1.5e9vg	-1.409	-2.299 to -0.5193	****	<0.0001
60 Days	WT Untreated vs. KO 3.75e9vg	-1.159	-1.920 to -0.3983	****	<0.0001
60 Days	WT Untreated vs. KO 7.5e9vg	-1.1	-1.940 to -0.2590	**	0.0011
60 Days	WT Untreated vs. KO 1.5e10vg	-0.8322	-1.612 to -0.05226	*	0.0242
60 Days	WT Untreated vs. KO 3.0e10vg	-1.455	-2.281 to -0.6279	****	<0.0001
60 Days	WT Untreated vs. KO 6.0e10vg	-1.359	-2.215 to -0.5034	****	<0.0001
90 Days	WT Untreated vs. KO Untreated	-4.088	-5.007 to -3.170	****	<0.0001
90 Days	WT Untreated vs. KO 1.5e9vg	-1.945	-3.593 to -0.2974	**	0.0062
90 Days	WT Untreated vs. KO 3.75e9vg	-1.792	-2.498 to -1.086	****	<0.0001
90 Days	WT Untreated vs. KO 7.5e9vg	-1.345	-1.943 to -0.7477	****	<0.0001
90 Days	WT Untreated vs. KO 1.5e10vg	-1.036	-1.614 to -0.4588	****	<0.0001
90 Days	WT Untreated vs. KO 3.0e10vg	-1.89	-2.512 to -1.268	****	<0.0001
90 Days	WT Untreated vs. KO 6.0e10vg	-2.279	-3.008 to -1.549	****	<0.0001

Table S3: Non-human Primate Injection Parameters

Material Injected	Animal ID	Age at Injection	IT Infusion Date	Bodyweight at Infusion (kg)	Viral Dose (vg)	Omnipaque™
scAAV9.P546.*MECP2*	15C38	12 Months	04/06/2016	1.68	$1.7x10^{13}$	0.4mL
scAAV9.P546. *MECP2*	15C49	12 Months	04/06/2016	1.30	$1.3x10^{13}$	0.4mL
scAAV9.P546. *MECP2*	15C40	12 months	04/06/2016	1.79	$1.6x10^{13}$	0.4mL
scAAV9.P546. *MECP2*	15C48	12 months	04/06/2016	1.83	$1.7x10^{13}$	0.4mL
scAAV9.P546. *MECP2*	15C34	6 months	10/23/2019	1.23	$9.2x10^{12}$	0.4mL