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Supplemental Information

rAAV8 and rAAV9-Mediated Long-Term Muscle Transduction with Tacrolimus (FK506) in Non-Human Primates

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Table S1

animals (vector/transgene)	Neutralizing Antibody	WBC($\times 10^6$ /ml)	Hb(g/dl)	PLT($\times 10^4$ /ml)	AST(IU/L)	ALT(IU/L)	ALP(IU/L)	CK(IU/L)	LDH(IU/L)	CRP(mg/dl)	Na(mEq/dl)	K(mEq/dl)	Cl(mEq/dl)	Ca(mg/dl)	tacrolimus(ng/ml)
No.1 (AAV8CMVLacZ)	negative	127.0 \pm 23.5	11.7 \pm 0.6	47.0 \pm 2.4	48.4 \pm 16.3	30.4 \pm 9.5	1317.4 \pm 272.9	210.0 \pm 160.9	1337.8 \pm 471.8	0.21 \pm 0.1	148.8 \pm 1.9	3.8 \pm 0.1	107.4 \pm 2.3	8.7 \pm 0.3	
No.2 (AAV8CMVLacZ) with tacrolimus	negative	95.8 \pm 23.0	11.7 \pm 0.5	41.9 \pm 5.4	55.6 \pm 21.1	123.6 \pm 71.0	1375.7 \pm 417.8	228.0 \pm 110.3	1091.7 \pm 289.6	0.08 \pm 0.02	146.8 \pm 1.2	3.9 \pm 0.0	107.7 \pm 2.1	9.3 \pm 0.3	17.7 \pm 2.2
No.3 (AAV9CMVLacZ)	negative	86.2 \pm 14.2	11.7 \pm 0.3	27.6 \pm 3.1	39.6 \pm 5.2	47.8 \pm 7.3	1605.6 \pm 109.3	192.6 \pm 58.8	192.6 \pm 257.9	0.04 \pm 0.01	147.2 \pm 1.3	3.9 \pm 0.1	108.8 \pm 0.8	9.0 \pm 0.4	
No.4 (AAV9CMVLacZ) with tacrolimus	AAV8 positive	100.0 \pm 17.2	12.8 \pm 0.5	44.6 \pm 2.5	61.0 \pm 10.2	24.4 \pm 4.3	4396.4 \pm 635.5	322.5 \pm 66.1	863.0 \pm 132.3	0.06 \pm 0.01	146.5 \pm 1.0	3.9 \pm 0.1	110.0 \pm 1.21	8.8 \pm 0.3	10.0 \pm 1.0
No.5 (AAV8CMVM3FLAG)	negative	84.0 \pm 14.7	11.7 \pm 0.2	39.8 \pm 0.9	89.4 \pm 13.2	144.2 \pm 35.0	1581.4 \pm 159.7	151.6 \pm 22.8	1522.2 \pm 322.6	0.06 \pm 0.0	146.8 \pm 1.3	3.5 \pm 0.2	107.2 \pm 0.8	9.4 \pm 0.2	
No.6 (AAV8CMVM3FLAG) with tacrolimus	negative	109.3 \pm 24.1	12.7 \pm 0.3	36.7 \pm 2.9	61.4 \pm 8.1	52.3 \pm 7.9	2203.0 \pm 100.0	282.8 \pm 40.5	1219.0 \pm 122.5	0.06 \pm 0.02	146.5 \pm 1.3	3.8 \pm 0.4	107.7 \pm 1.6	8.6 \pm 0.4	17.8 \pm 1.2
No.7 (AAV9CMVM3FLAG)	negative	144.8 \pm 24.6	13.2 \pm 0.5	38.2 \pm 3.8	61.6 \pm 15.6	69.6 \pm 8.1	3306.2 \pm 451.6	253.0 \pm 52.3	1117.4 \pm 466.2	0.06 \pm 0.01	147.8 \pm 0.8	4.3 \pm 0.5	105.8 \pm 2.2	9.4 \pm 0.4	
No.8 (AAV9CMVM3FLAG) with tacrolimus	negative	76.1 \pm 11.2	11.6 \pm 0.5	31.8 \pm 2.6	62.8 \pm 17.0	204.4 \pm 80.8	1269.3 \pm 287.3	210.1 \pm 69.8	502.9 \pm 111.1	0.09 \pm 0.04	148.1 \pm 1.9	3.4 \pm 0.1	109.7 \pm 2.1	8.2 \pm 0.4	13.0 \pm 1.3

Table S2

anti-AAV2	anti-AAV8	anti-AAV9	Number of monkeys
-	-	-	9 (36%)
+	-	-	2 (8%)
-	+	-	7 (28%)
+	+	-	1 (4%)
+	+	+	6 (24%)

Fig.S1

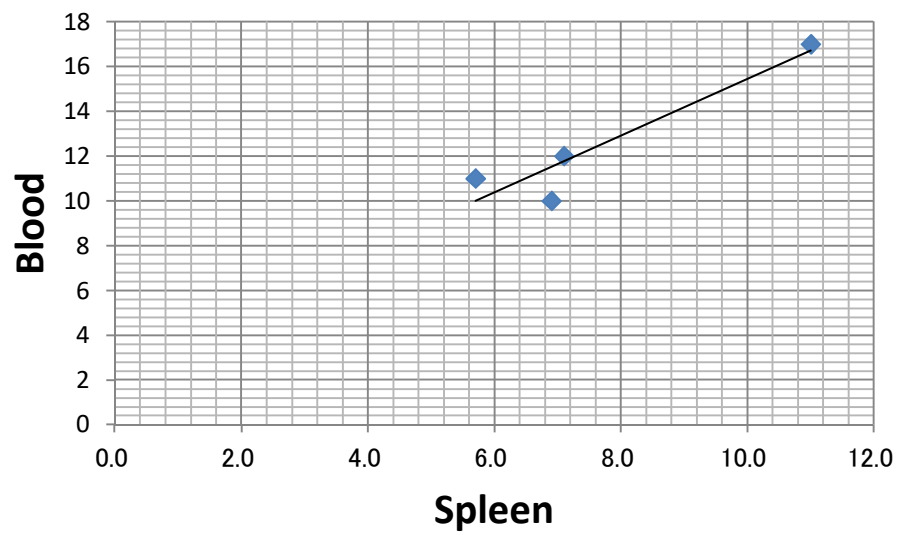
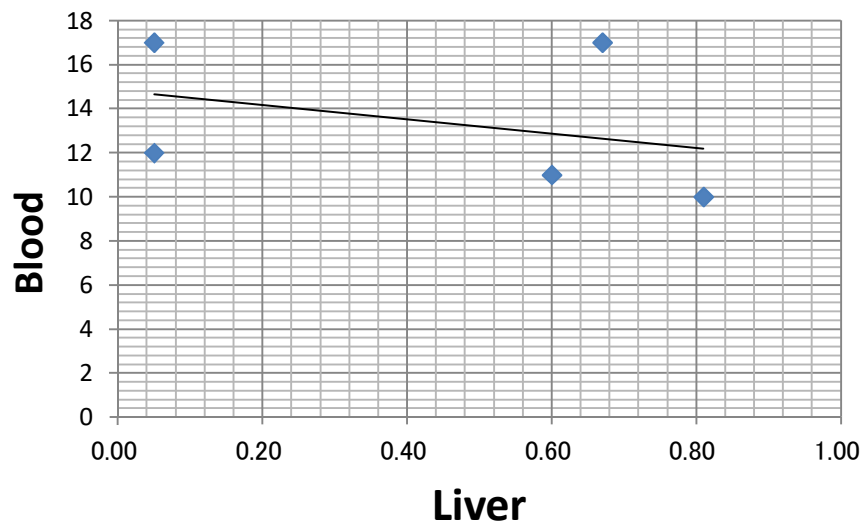


Fig. S2

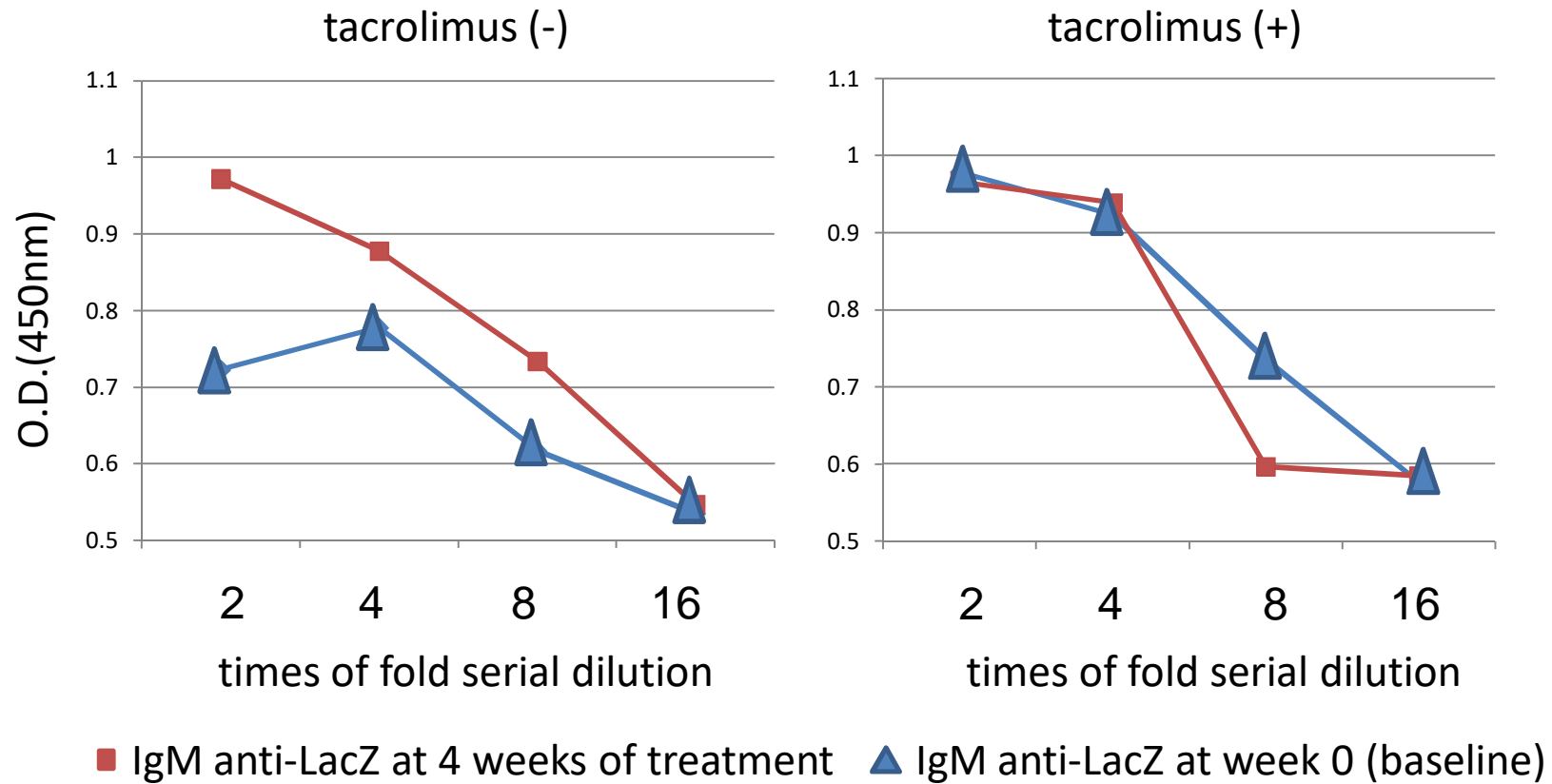


Figure Legends

Table S1: Blood laboratory data of treated monkeys during the experiment

During the experiment, no significant and critical change was observed.

No monkeys showed laboratory abnormalities or morbidity during the observation period.

Table S2: AAV-neutralizing antibody (cell-based assay) screening results.

Of the 25 monkeys examined, 9 did not have antibodies for AAV2, AAV8, nor AAV9, two monkeys had antibodies against AAV2, seven had antibodies against AAV8, and one had antibodies against both AAV2 and AAV8.

Six had antibodies for all AAV2, AAV8, and AAV9; no monkeys had antibodies against AAV9.

In total, 64% of the monkeys were seropositive against at least one serotype of AAV.

Fig. S1

Concentration of tacrolimus in the liver and spleen

Concentration of tacrolimus in the blood was below 20 ng/mL throughout the experiment.

Concentrations in the blood and spleen (approximately expression, $y = 1.2683x + 2.7659$, decision coefficient $R^2 = 0.8812$) were better correlated compared to in the blood and liver ($y = -3.2184x + 14.803$, $R^2 = 0.1191$).

Fig. S2

IgM antibody for LacZ with or without tacrolimus treatment

Without tacrolimus, IgM for LacZ was produced for 4 weeks (square) after AAV9CMVLacZ injection.

In contrast, there was no IgM for LacZ after AAV9CMVLacZ injection when tacrolimus co-treatment was used.

Triangle shows baseline (0 weeks).