

### Supplementary Materials for

### Follistatin Gene Delivery Enhances Muscle Growth and Strength in Nonhuman Primates

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Table S1. Menstrual cycles in untreated control and AAV1-FS344-treated cynomolgus macaques.

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Fig. S1

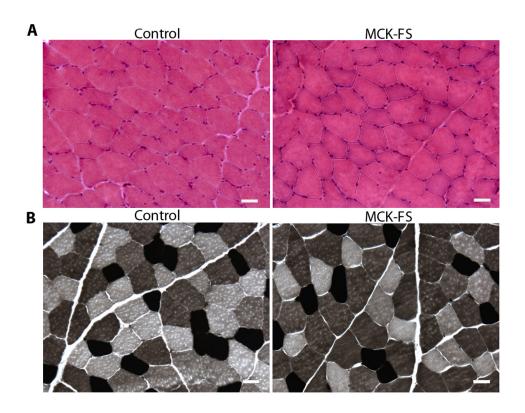


Fig. S1 MCK-FS causes myofiber hypertrophy and predominantly affects fast-twitch type 2 myofibers in the quadriceps muscle.

(A) H&E staining of the quadriceps muscle reveals myofiber hypertrophy in MCK-FS macaque (right) compared to untreated control (left). (B) Fiber types determined by ATPase staining (pH 4.6) in the MCK-FS macaque (right) compared to untreated control (left). Scale bars, 20µm.

Table S1

Months Post Injection	Control	MCK-FS	CMV-FS
10 months	+ (27)	+ (26)	+ (26)
11 months	+ (24)	+ (31)	+ (28)
12 months	+ (28)	+ (24)	ND
13 months	ND	+ (29)	+
14 months	+	+ (31)	+ (30)
15 months	+ (29)	+	+ (26)
Mean ± SD menstrual cycle duration (days)	27.0 ± 2.2	28.2 ± 3.1	27.5 ± 1.9

Abbreviations: ND, Not detected; SD, Standard Deviation

## Table S1 Menstrual cycles in untreated control and AAV1-FS344 treated cynomolgus macaques.

Menstrual cycles were monitored in female macaques following gene transfer. Both CMV-FS and MCK-FS treated macaques showed normal menstrual cycle duration compared to untreated control macaque. Data from 6 months prior to necropsy are shown.

<sup>&</sup>quot;+" indicates menstrual cycle detected. A value in parenthesis indicates days between two consecutive menstrual cycles.

Table S2

Group	Motility (%)	Normal Morphology (%)	Abnormalities (%)
Control #1	60	91	9
Control #2	90	77	23
Mean (Control)	75	84	16
CMV-FS	65	76	24

# Table S2 Sperm motility and morphology of untreated control and AAV1-FS344 treated cynomolgus macaques.

Prior to necropsy, sperm was collected from untreated control and AAV1-FS344 treated macaques. There was no significant change in sperm motility and morphology in the CMV-FS macaque compared to untreated control macaques.

Fig. S2

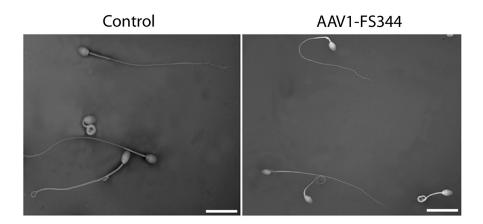


Fig. S2 Morphological effects on sperm from male cynomologus macaques treated with AAV1-FS344.

Prior to necropsy, sperm was collected from untreated control and AAV1-FS344 treated macaques. The morphology of CMV-FS treated macaque sperm is similar to untreated controls.

Table S3

Amplicon	Forward primer	Reverse primer	Taqman probe
	sequence	sequence	sequence
CMV	5'TGGAAATCCCCGTG	5'CATGGTGATGCGG	5'CCGCTATCCACGC
amplicon	AGTCAA3'	TTTTGG3'	CCATTGATG3'
MCK	5'CCCGAGATGCCTGG	5'GCTCAGGCAGCAG	5'CCAGACATGTGGC
amplicon	TTATAATT3'	GTGTTG3'	TGCTCCCCC3'

Table S3 Primer and probe sets used in quantitative polymerase chain reaction (QPCR) for vector genome quantification.