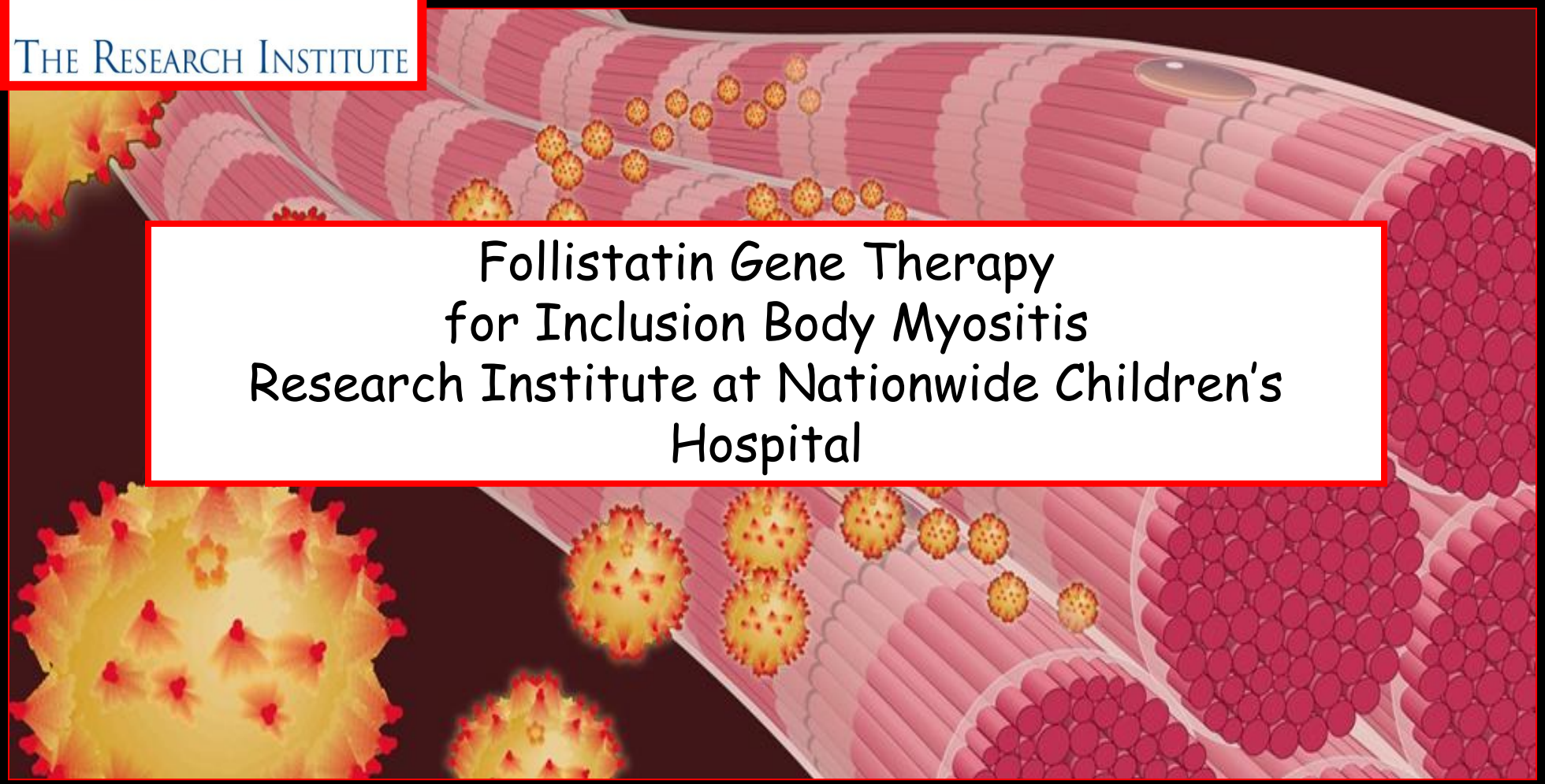
NATIONWIDE CHILDREN'S HOSPITAL℠

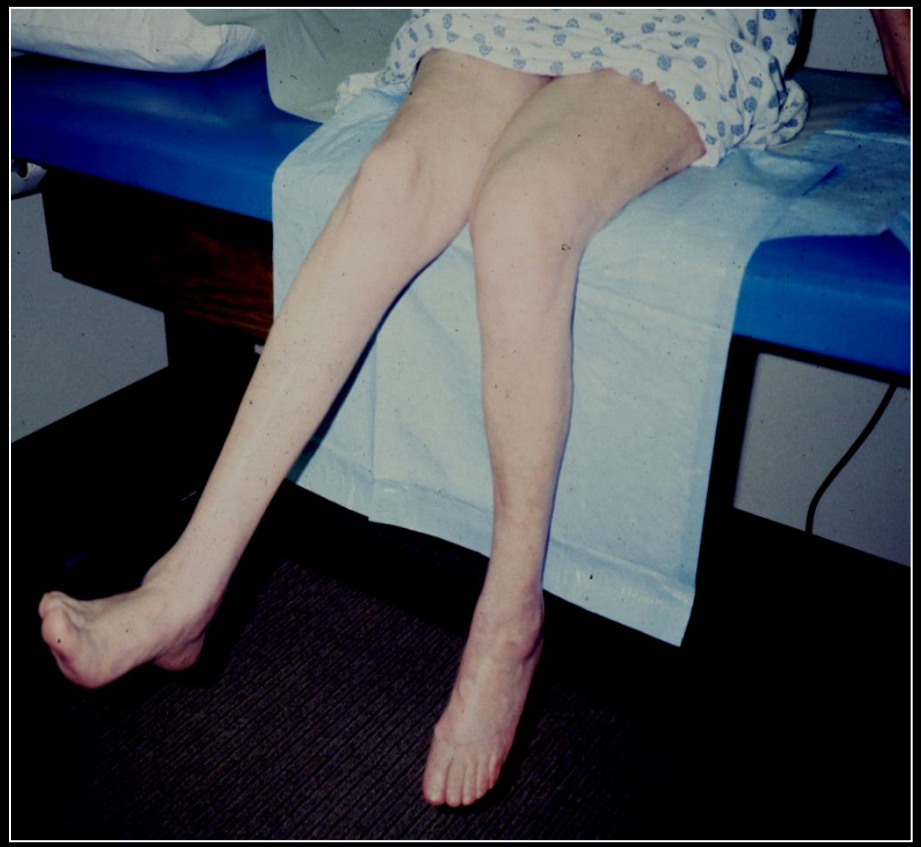
THE RESEARCH INSTITUTE



# Follistatin Gene Therapy for Inclusion Body Myositis Research Institute at Nationwide Children's Hospital

# The Clinical Problem in sIBM

- Quadriceps muscle weakness
- Genu recurvatum (pain)
- Frequent falls
  - Limb fractures
- A clinical trial improving quadriceps muscle strength would result in a "clinically meaningful outcome"



# Resistant to other Approaches

- Weight training
- Electrical Stimulation
- Anabolic Steroids

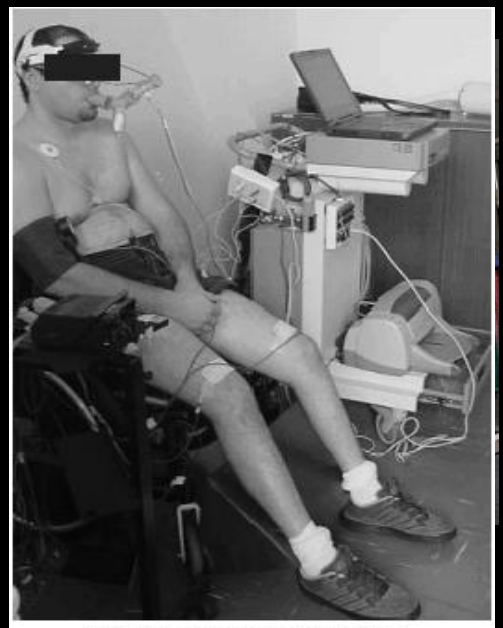
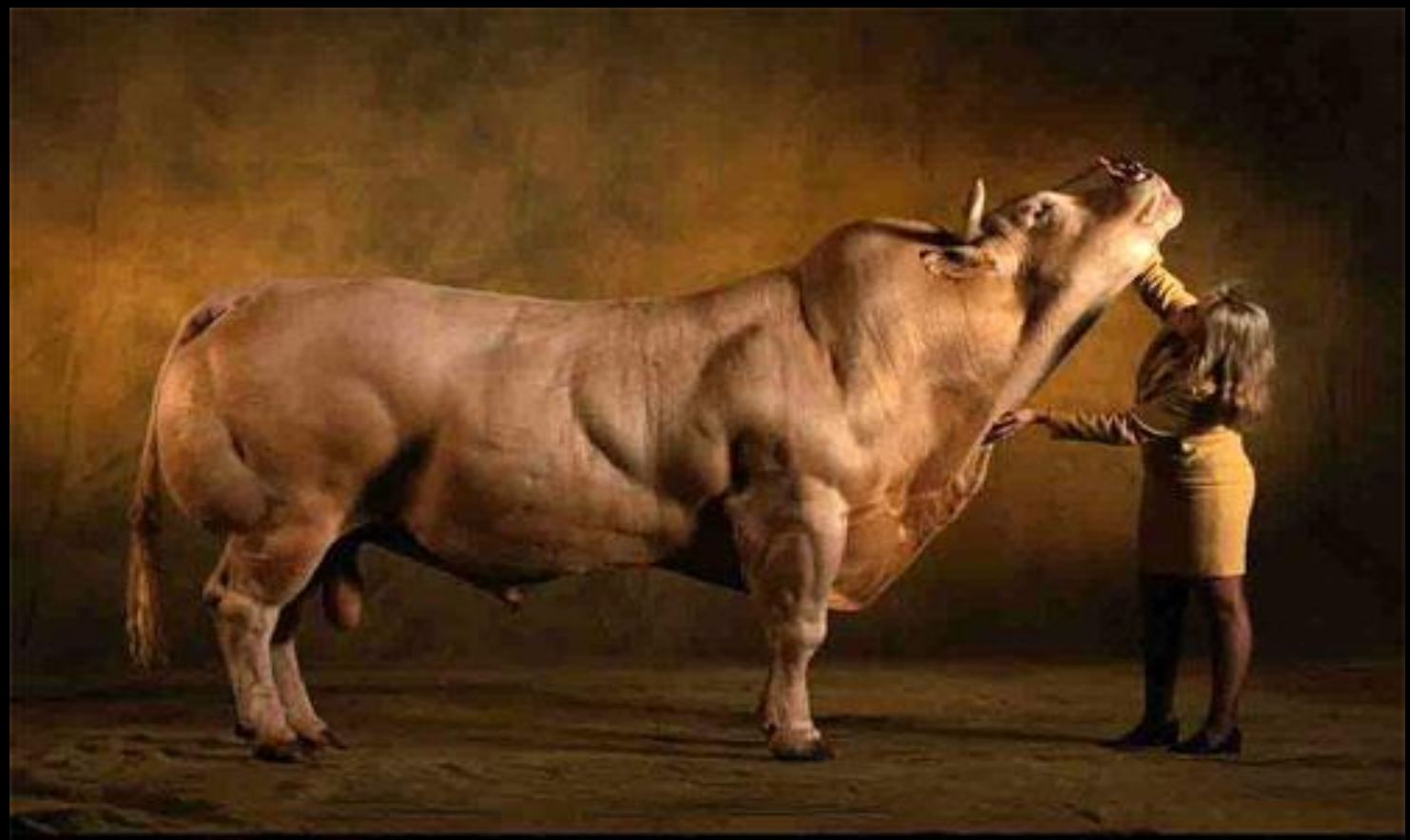
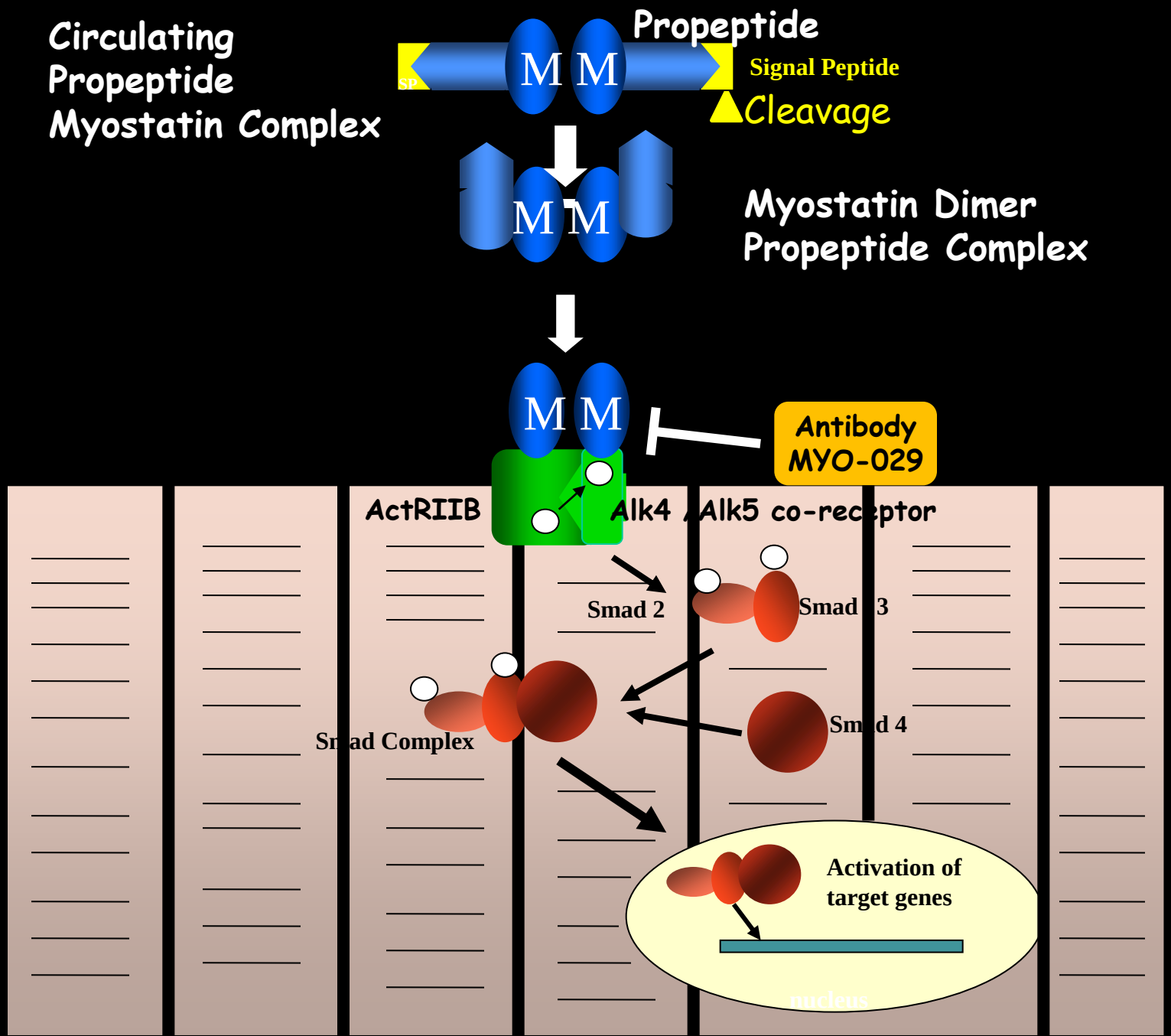


*Figure 1 - Experimental procedure*

# Myostatin Inhibition in the Cattle Industry



Can Muscle Enhancing Strategies be effective in neuromuscular disorders (Myositis, Muscular Dystrophy, SMA, ALS?)


Circulating
Propeptide
Myostatin Complex
Propeptide
SP
M M
Signal Peptide
Cleavage
M M
Myostatin Dimer
Propeptide Complex
M M
Antibody
MYO-029
ActRIIB
Alk4 / Alk5 co-receptor
Smad 2
Smad 3
Smad Complex
Smad 4
Activation of
target genes
nucleus

ORIGINAL ARTICLE

Ann Neurology March 11, 2008

# A Phase I/II trial of MYO-029 in Adult Subjects with Muscular Dystrophy

Kathryn R. Wagner, MD, PhD,[1] James L. Fleckenstein, MD,[2] Anthony A. Amato, MD,[3] Richard J. Barohn, MD,[4] Katharine Bushby, MD,[5] Diana M. Escolar, MD,[6] Kevin M. Flanigan, MD,[7] Alan Pestronk, MD,[8] Rabi Tawil, MD,[9] Gil I. Wolfe, MD,[10] Michelle Eagle, PhD, MSc, MCSP, SRP,[5] Julaine M. Florence, PT, DPT,[8] Wendy M. King, PT,[11] Shree Pandya, MS, PT,[9] Volker Straub, MD,[5] Paul Juneau, MS,[12] Kathleen Meyers, RN, BSN,[13] Cristina Csimma, PharmD, MHP,[14] Tracey Araujo, MSPharm,[14] Robert Allen, MD,[13] Stephanie A. Parsons, PhD,[13] John M. Wozney, PhD,[14] Edward R. LaVallie, PhD,[14] and Jerry R. Mendell, MD[11]

- Wyeth sponsored 11 Center Trial (10 USA;1GB) Using MYO-029 antibody to myostatin
  - No Clinical Benefit
  - Muscle histology showed a trend toward increased muscle fiber size
  - Demonstrated safety of systemic delivery of a myostatin inhibitor in a clinical trial

# Natural inhibitor proteins of myostatin leads to enlarged muscles

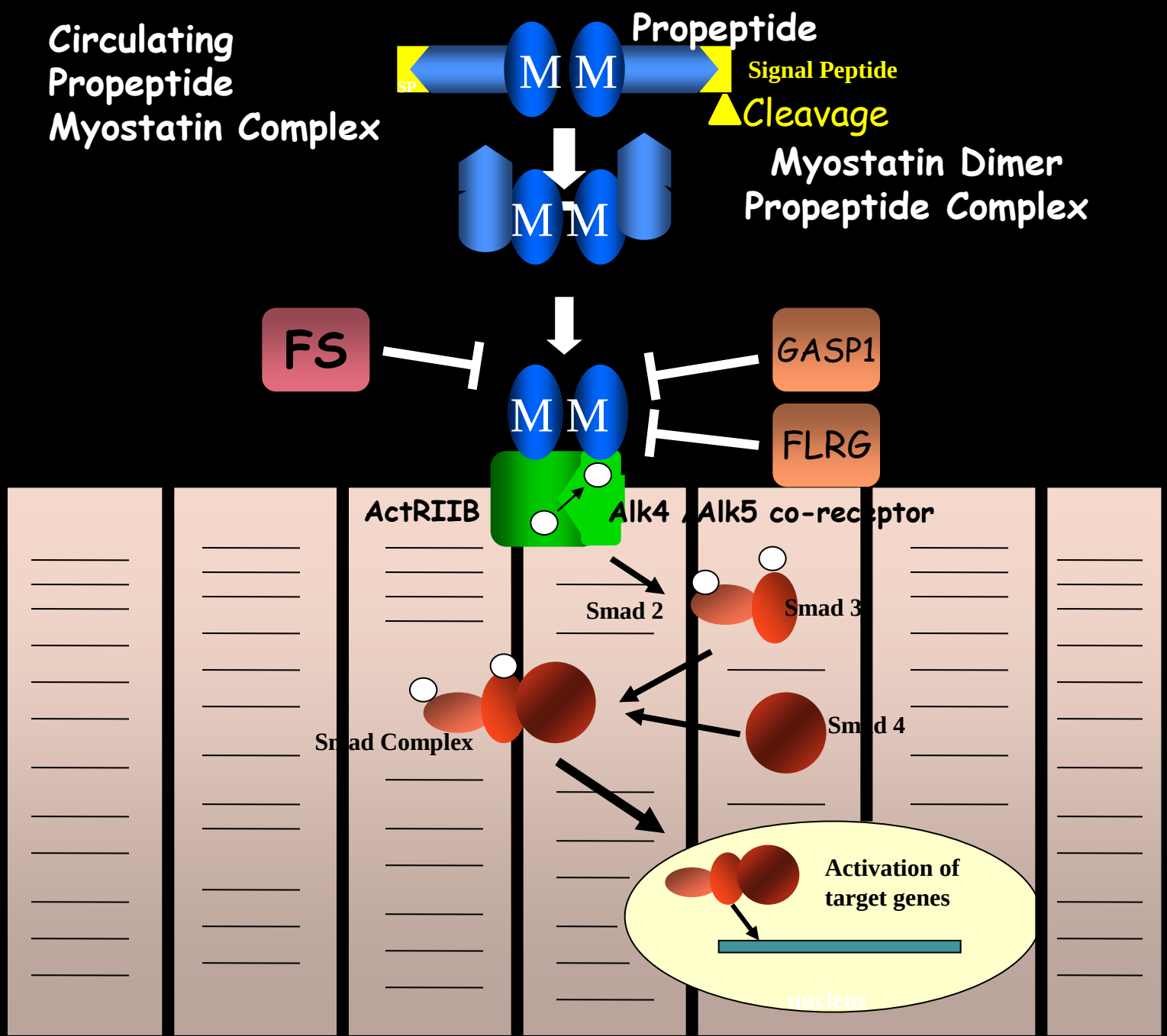
3 Known Myostatin Inhibiting Proteins

Follistatin
Follistatin like related gene (FLRG)
Growth and serum protein (GASP1)

Wildtype

Follistatin over-expression

Lee, S.J. & A.C. McPherron PNAS (2001).

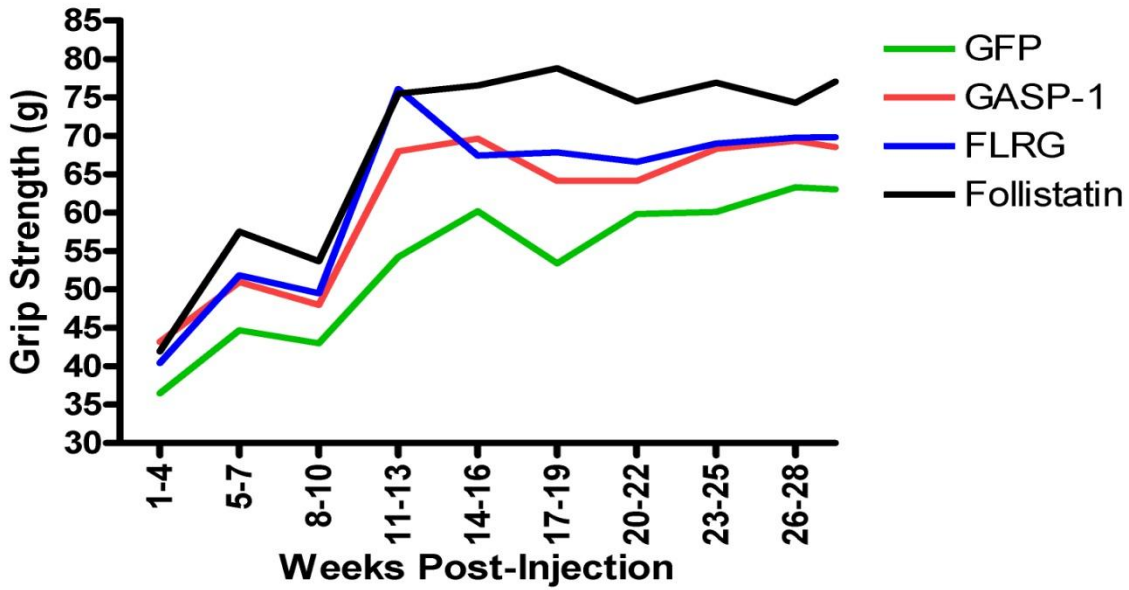

Circulating
Propeptide
Myostatin Complex
Propeptide
SP
M M
Signal Peptide
Cleavage
Myostatin Dimer
Propeptide Complex
M M
FS
GASP1
FLRG
M M
ActRIIB
Alk4 / Alk5 co-receptor
Smad 2
Smad 3
Smad Complex
Smad 4
Activation of
target genes
nucleus

# Candidate Genes for Myostatin Inhibition

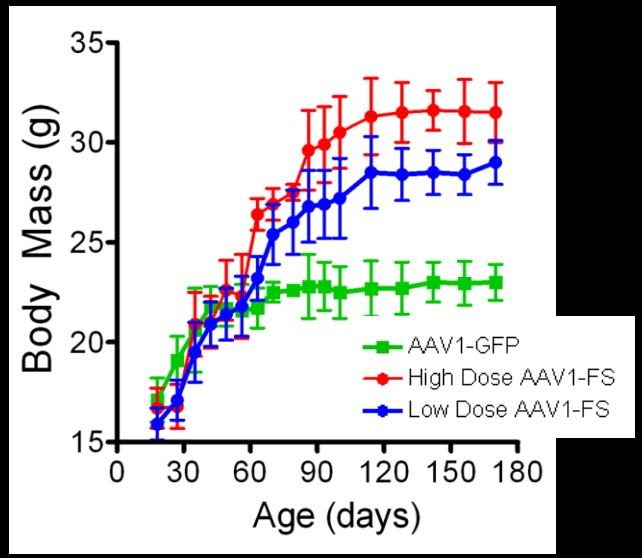
## Kaspar Lab Center for Gene Therapy

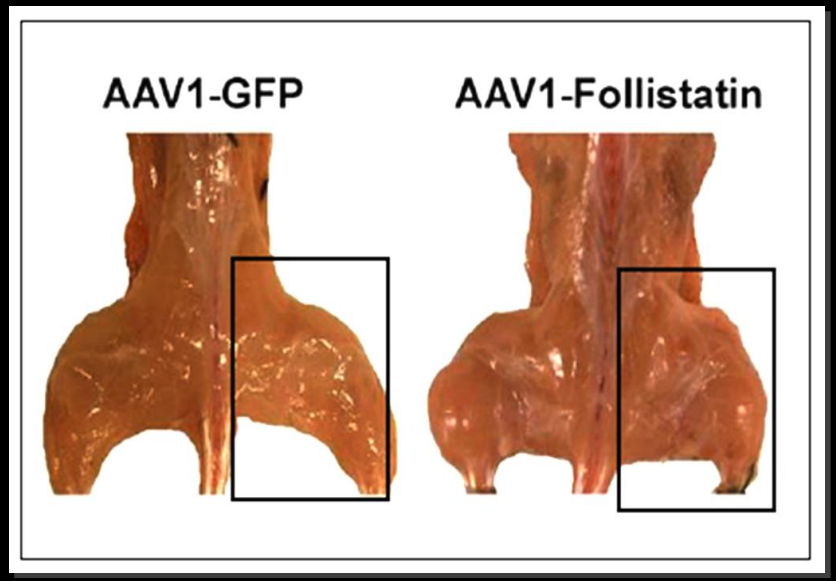
- Three candidate genes follistatin, FLRG, & GASP-1
- Follistatin performed better in head-to-head comparison when expressed by rAAV

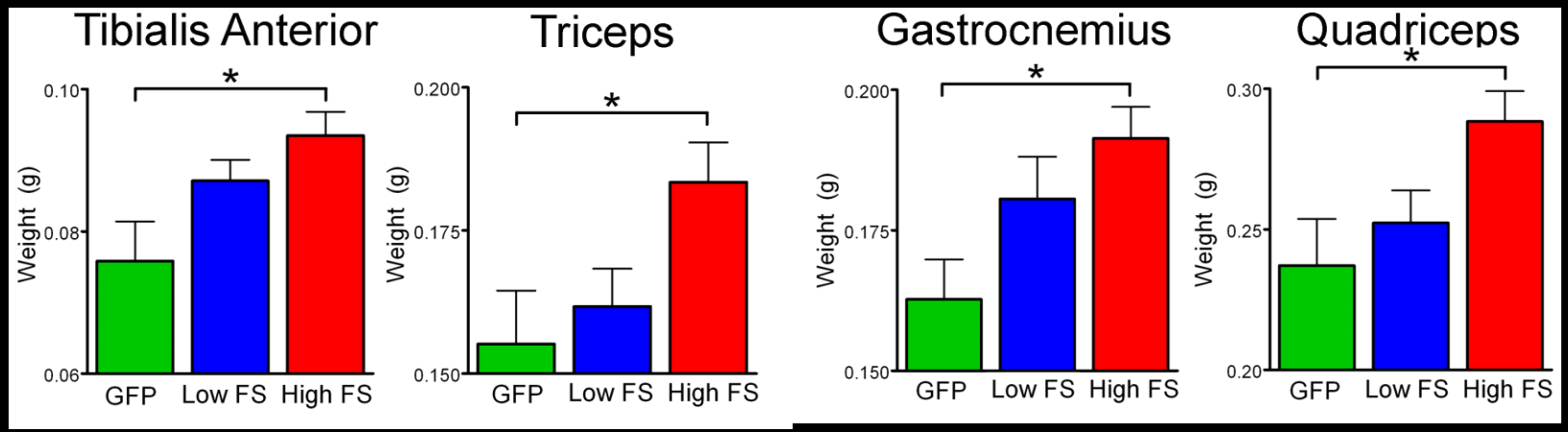
Comparison of hindlimb Strength in C57/B6 mice



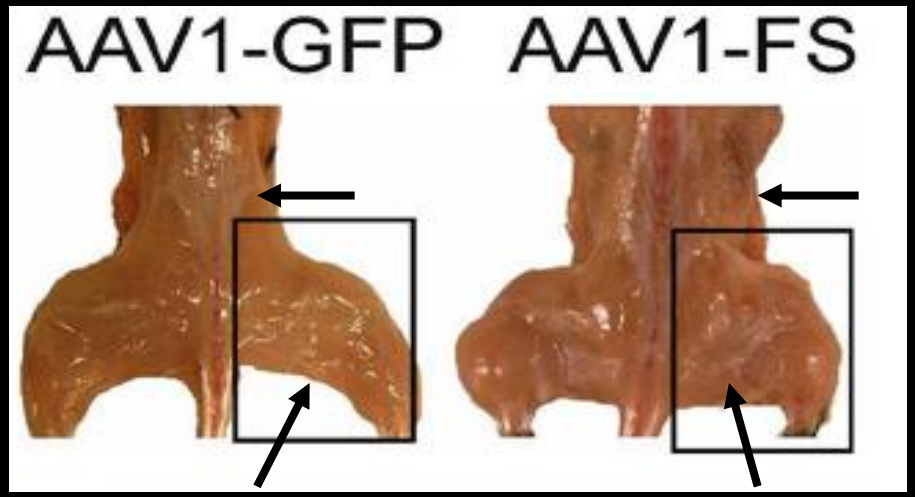
# AAV1-Follistatin increases overall body mass and individual muscle weights in mdx mice



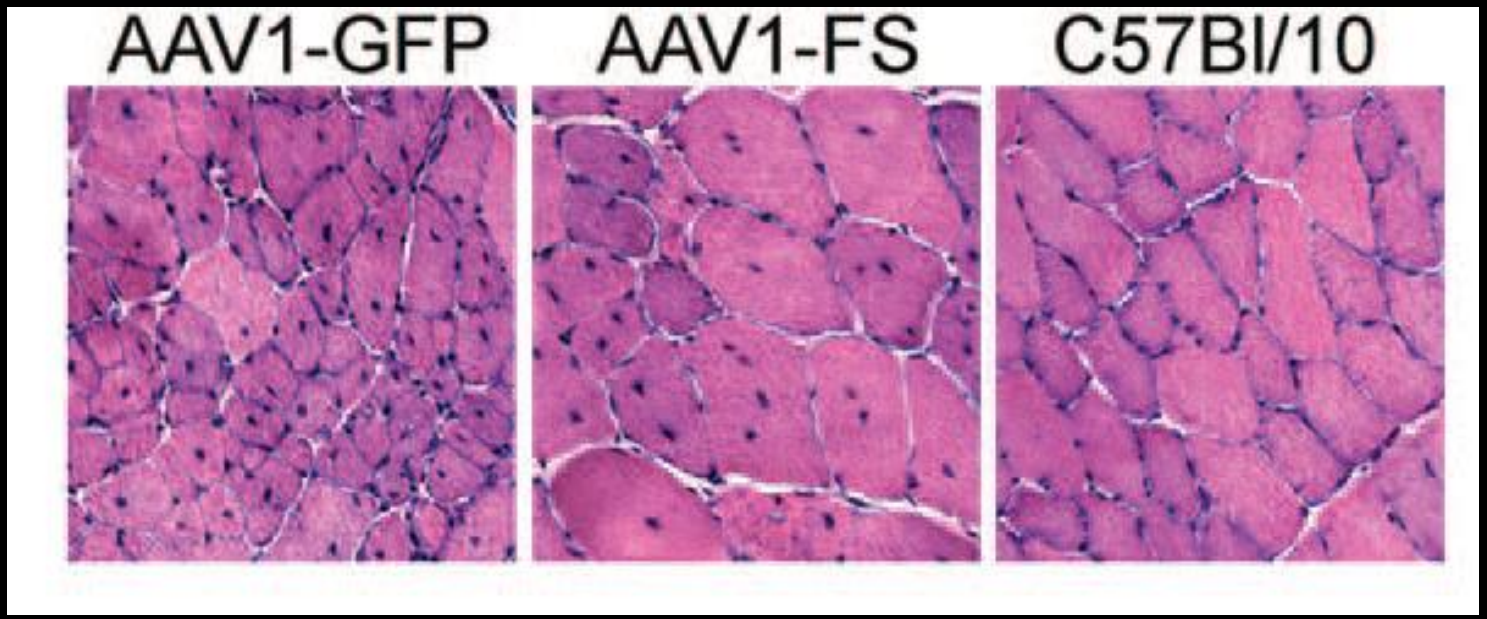
# Treatment of mdx mouse



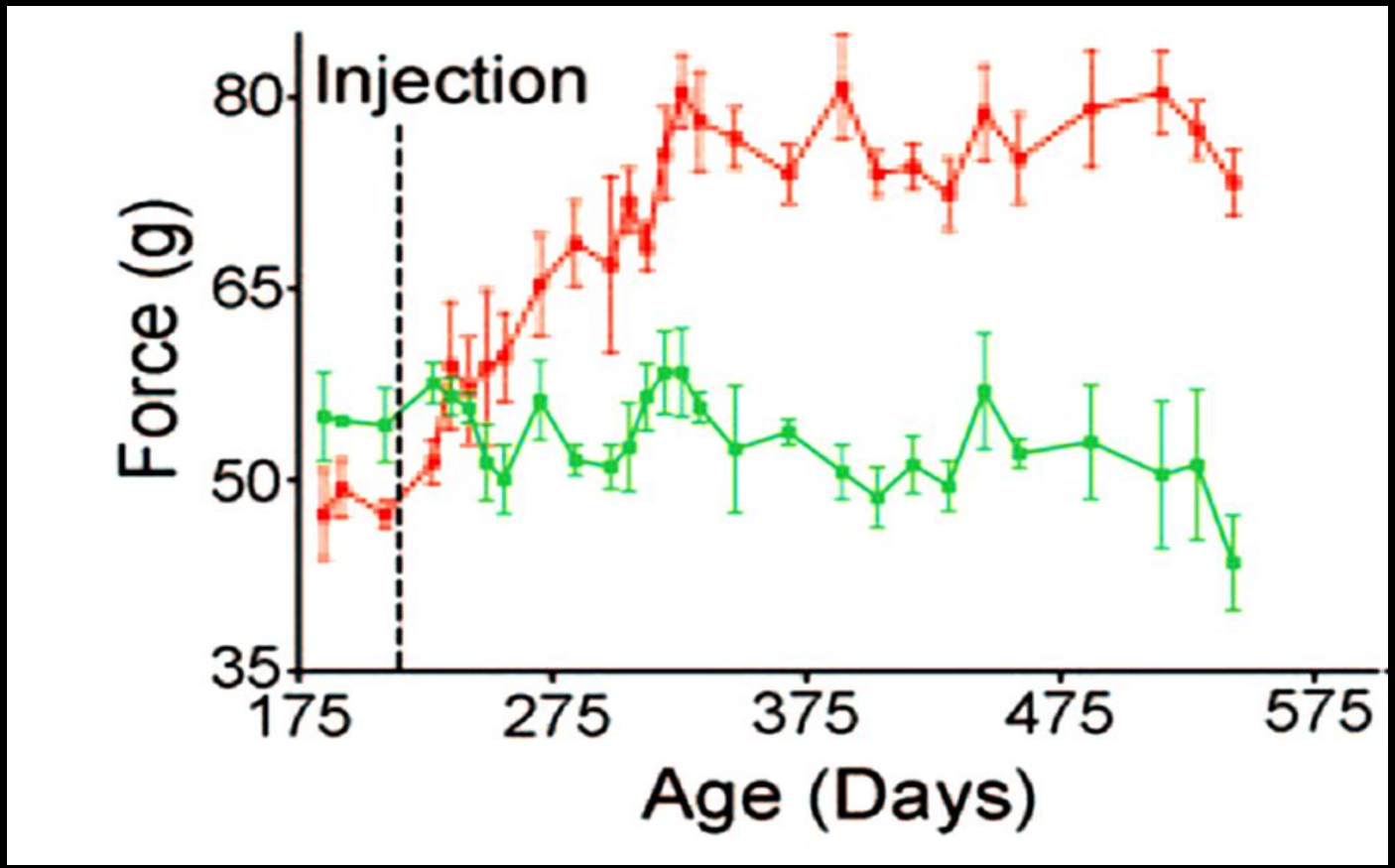

Injection of Leg Muscle

AAV1-FS344

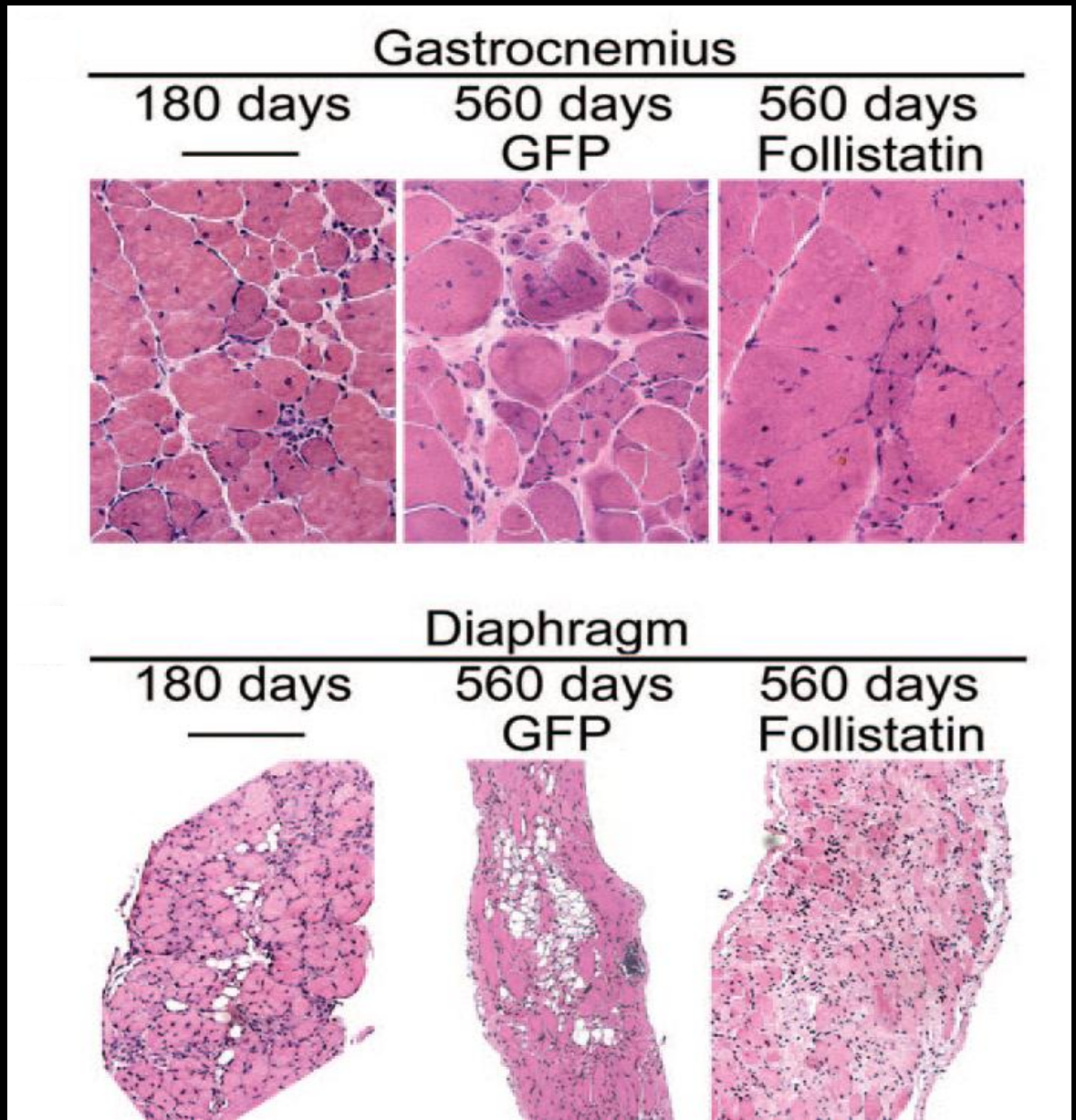
180 days



# AAV1-Follistatin Increases Strength in aged *mdx* animals



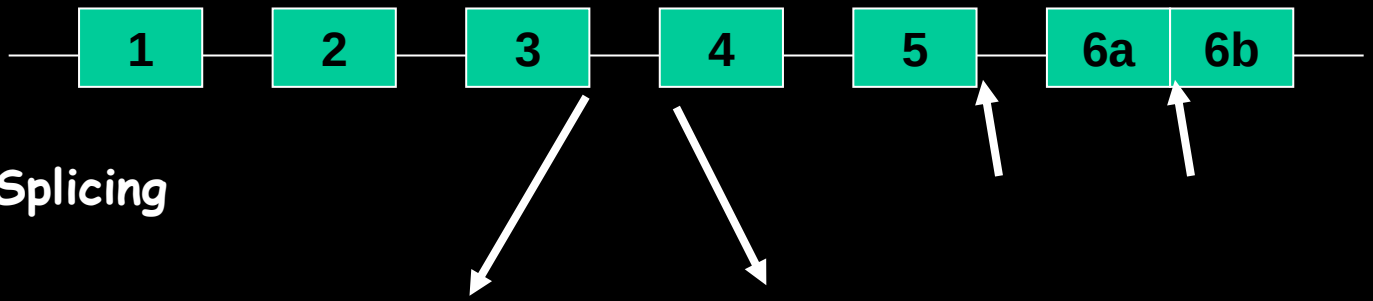
# Aged-mdx animals display reduced pathology when Treated with AAV-Follistatin at 210 days of age



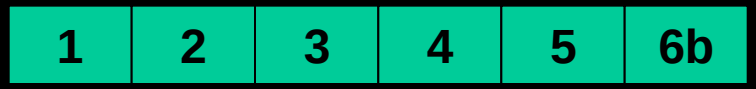
# Fulfilling Criteria for an IND

- Can transgene expression escape reproductive complications ?
  - FS binds activins and suppresses synthesis follicle-stimulating (FSH) at gonadal and Hypothalamic/Pit sites
- Can FS demonstrate efficacy in building muscle size and strength ?
- Is there functional improvement ?

# Follistatin Gene




Alternative Splicing



FS317

29 AA

Tissue bound Peptide FS 288



FS344

29 AA

Serum Peptide FS 315


10 X affinity for activin receptor

# Reproductive Data in Mice Used in Pre-clincal Studies

Reproductive Study (C57Bl/10)

| Group | Mean Litter Size (SD) |
|---|---|
| AAV1-FS Male Treated x Untreated Female (n=4) | 9.0 (2.582) |
| AAV1-FS Female Treated x Untreated Male (n=4) | 9.25 (1.708) |
| Untreated Male x Untreated Female (n=4) | 9.0 (2.160) |

Reproductive Study (*mdx*)

| Group | Mean Litter Size (SD) |
|---|---|
| AAV1-FS Male Treated x Untreated Female (n=3) | 4.5 (0.707) |
| AAV1-FS Female Treated x Untreated Male (n=2) | 2.0 (0) |
| Untreated Male x Untreated Female (n=6) | 3.83 (1.169) |

# Follicle Stimulating Hormone in Mice

| | 4 wks | 12wks | 20wks |
|---|---|---|---|
| 1763 | 2.56 | 14.2 | 13.64 |
| 1764 | 13.44 | 2 | 2.65 |
| 1765 | 2.78 | 1.68 | 4.56 |
| 1766 | 2.11 | 2.43 | 1.05 |
| 1767 | 2.56 | 4.85 | 1.35 |
| 1741 | 2.43 | 2.25 | 1.54 |
| 1743 | 4.58 | 1.82 | 10.81 |
| 1744 | 3.16 | 2.28 | 2.06 |
| 1745 | 9.33 | 2.15 | 2.81 |
| 1758 | 20.02 | 22.63 | 19.84 |
| 1759 | 23.14 | 21.71 | 18.55 |
| 1760 | 22.38 | 21.92 | 22.7 |
| 1761 | 17.68 | 21.18 | 19.69 |
| 1762 | 17.64 | 23.23 | 20.51 |
| 1746 | 18.42 | 18.84 | 17.63 |
| 1771 | 18.84 | 23.09 | 21.95 |
| 1748 | 18.35 | 16.04 | 19.32 |
| 1749 | 17.8 | 23.13 | 23.88 |
| 1750 | 18.33 | 18.54 | 22.87 |

Female Controls

4.650 ± 5.2

Female FS

4.305 ± 4.3

Male Controls

20.258 ± 1.5

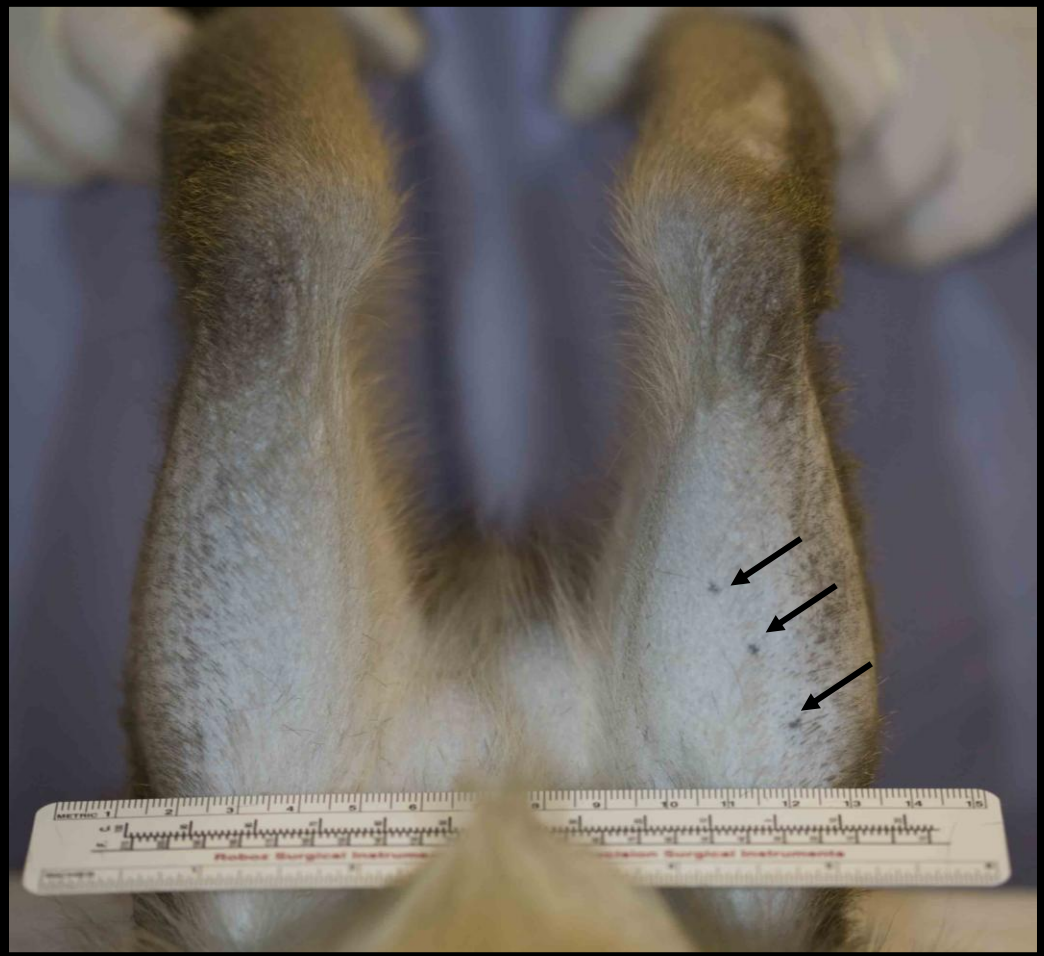
Male FS

21.13 ± 2.6

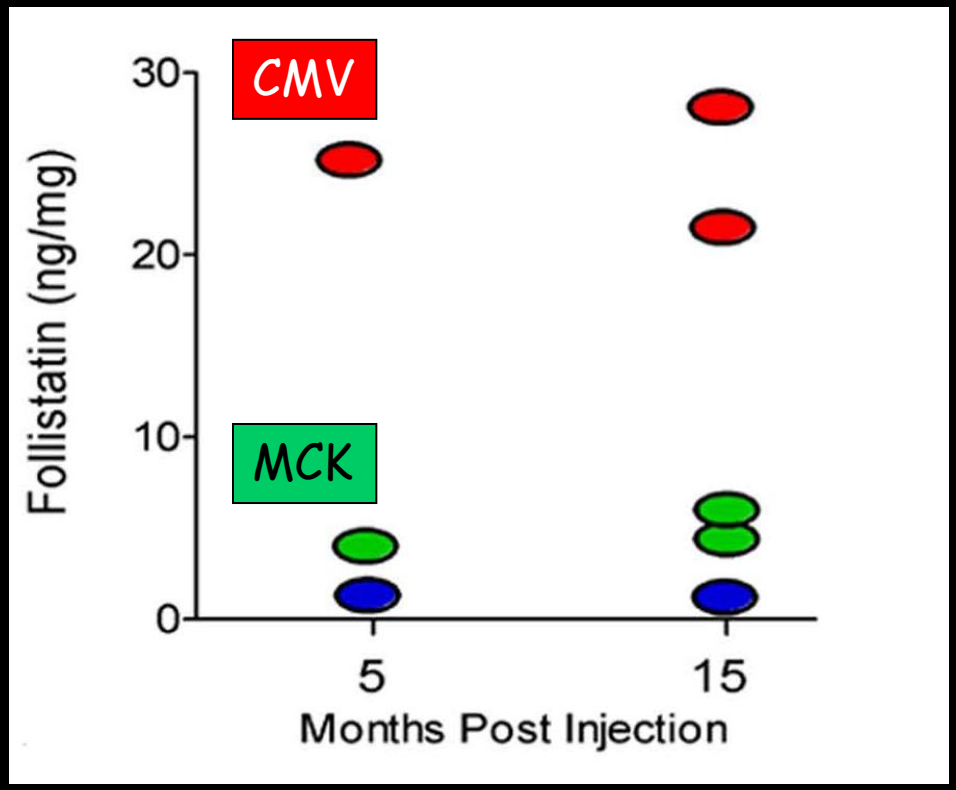
Can Mouse Studies Predict Efficacy in a Clinical Trial ?

MOVING TO NON-HUMAN PRIMATE

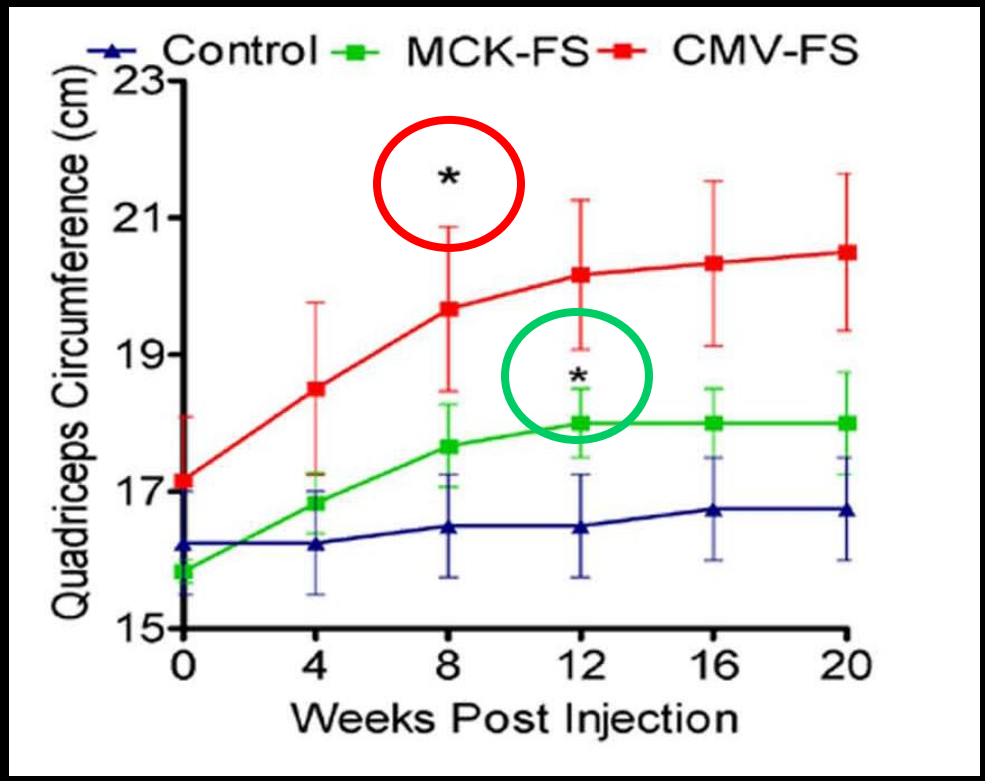
# FS344 Gene Transfer to Monkey

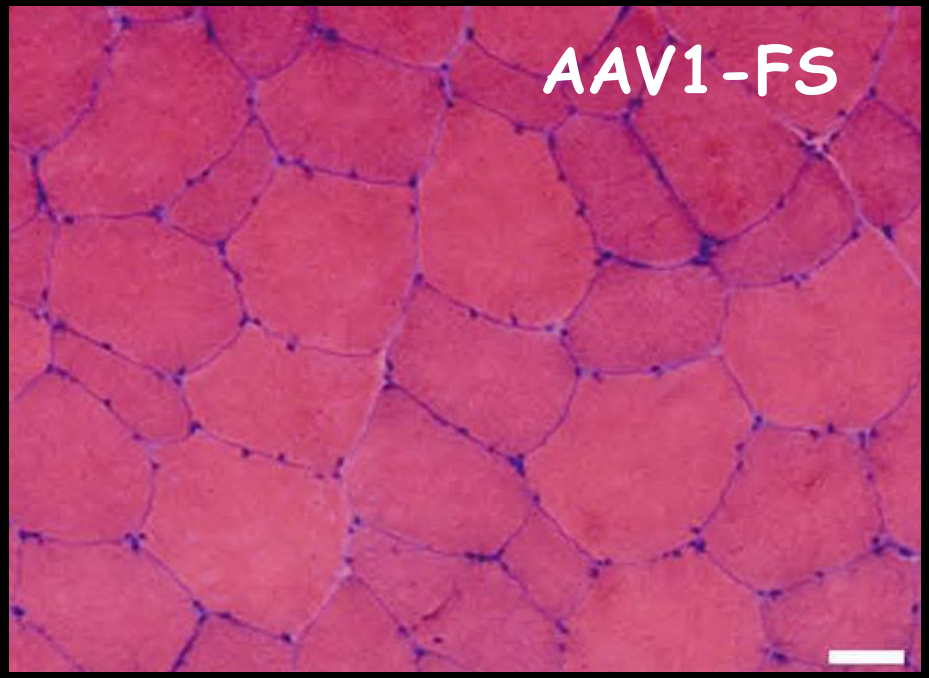


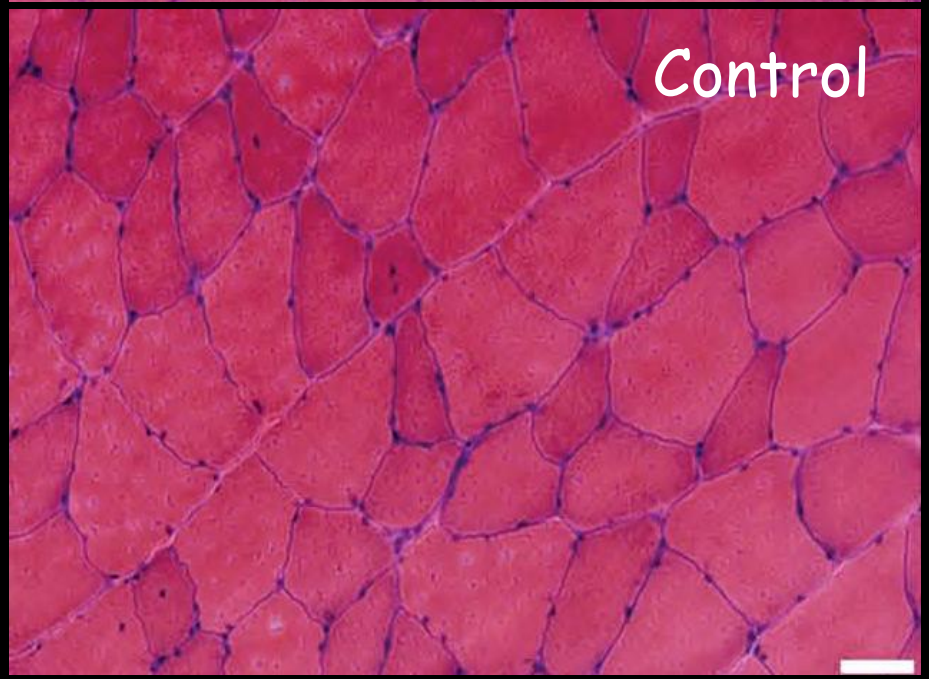
## FS Serum Levels

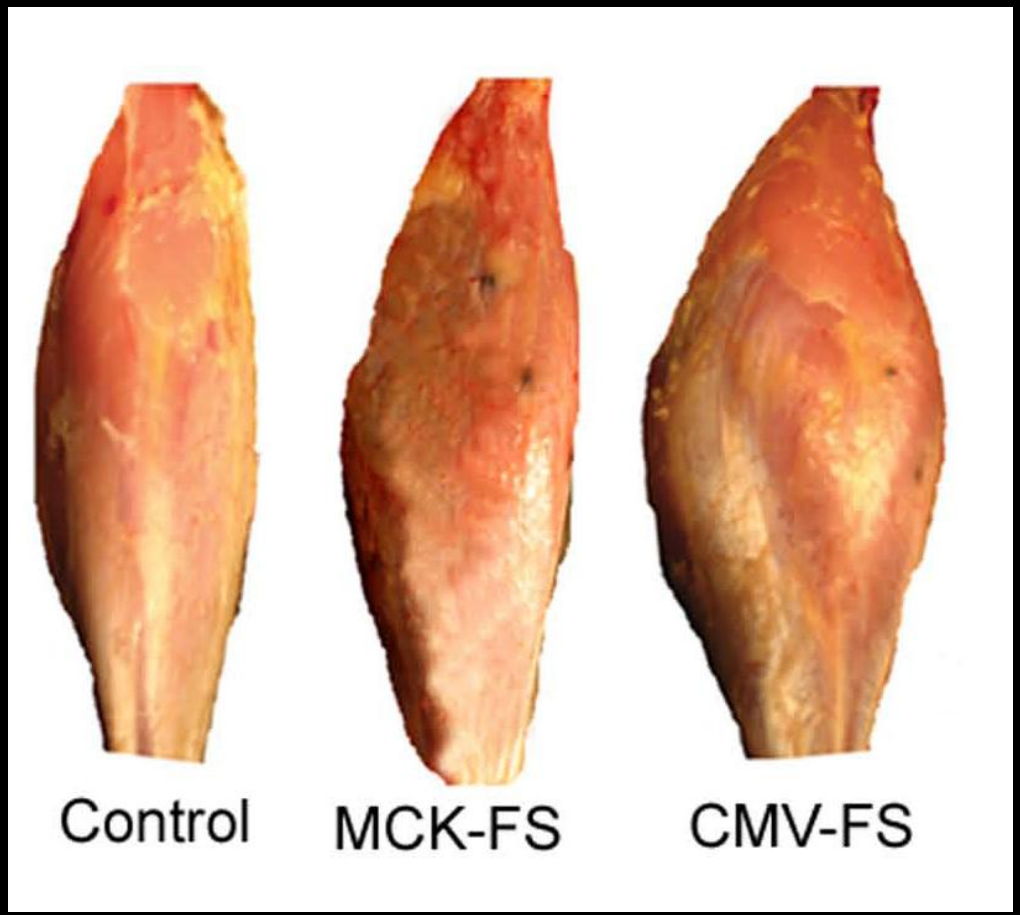


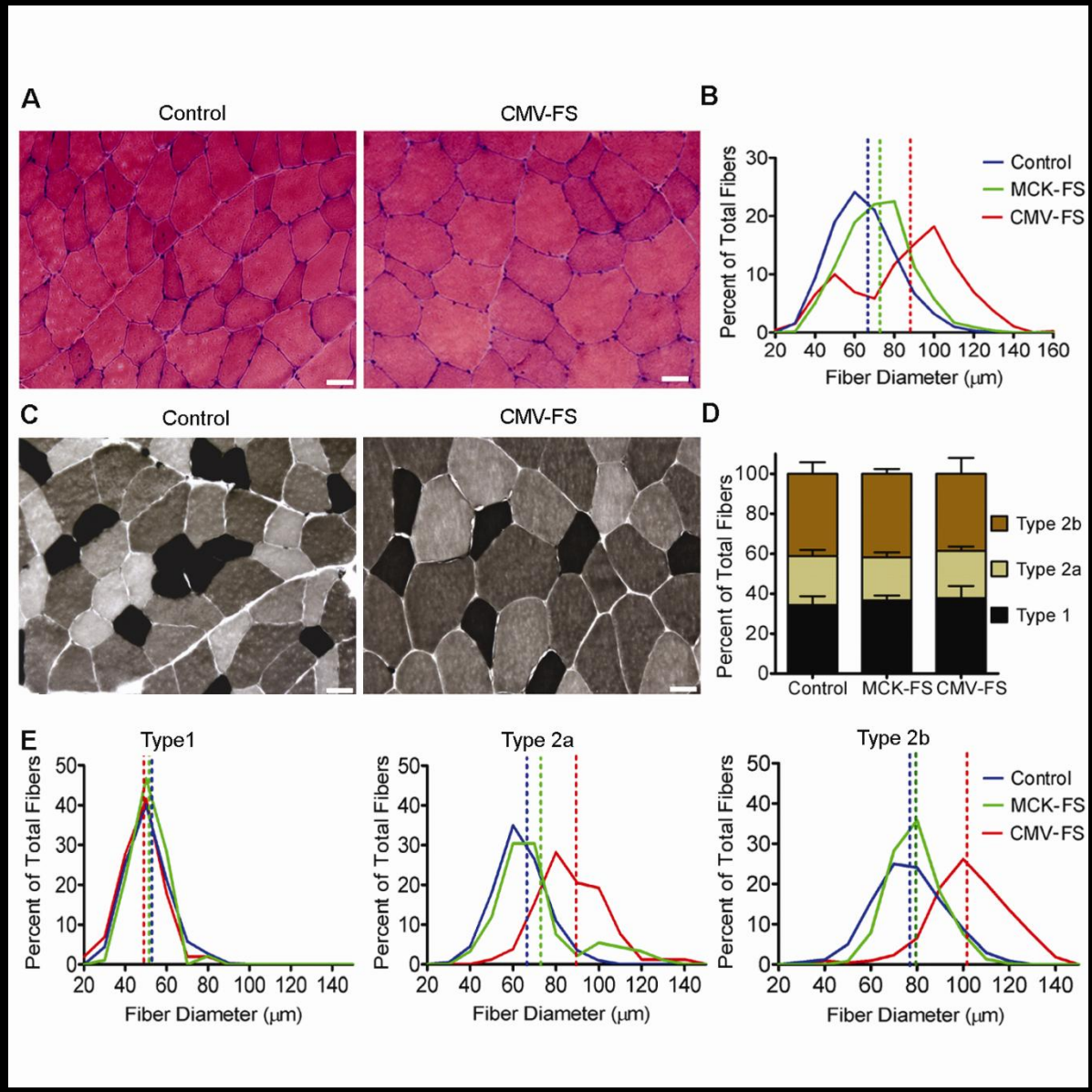

## Thigh Circumference




AAV1-FS


Control


Control
MCK-FS
CMV-FS


A
Control
CMV-FS
B
Percent of Total Fibers
Control
MCK-FS
CMV-FS
Fiber Diameter (μm)
C
Control
CMV-FS
D
Percent of Total Fibers
Type 2b
Type 2a
Type 1
Control
MCK-FS
CMV-FS
E
Type1
Type 2a
Type 2b
Percent of Total Fibers
Fiber Diameter (μm)

# Functional Improvement

| Promoter | Twitch Force | | Tetanic Force | |
|---|---|---|---|---|
| | Untreated Leg | FS-Treated | Untreated leg | FS-Treated |
| MCK | 17.0 | 19.0 (11.8%) | 65.0 | 73.0 (12.3%) |
| CMV | 19.0 | 24.0 (26.3%) | 64.0 | 72.0 (12.5%) |

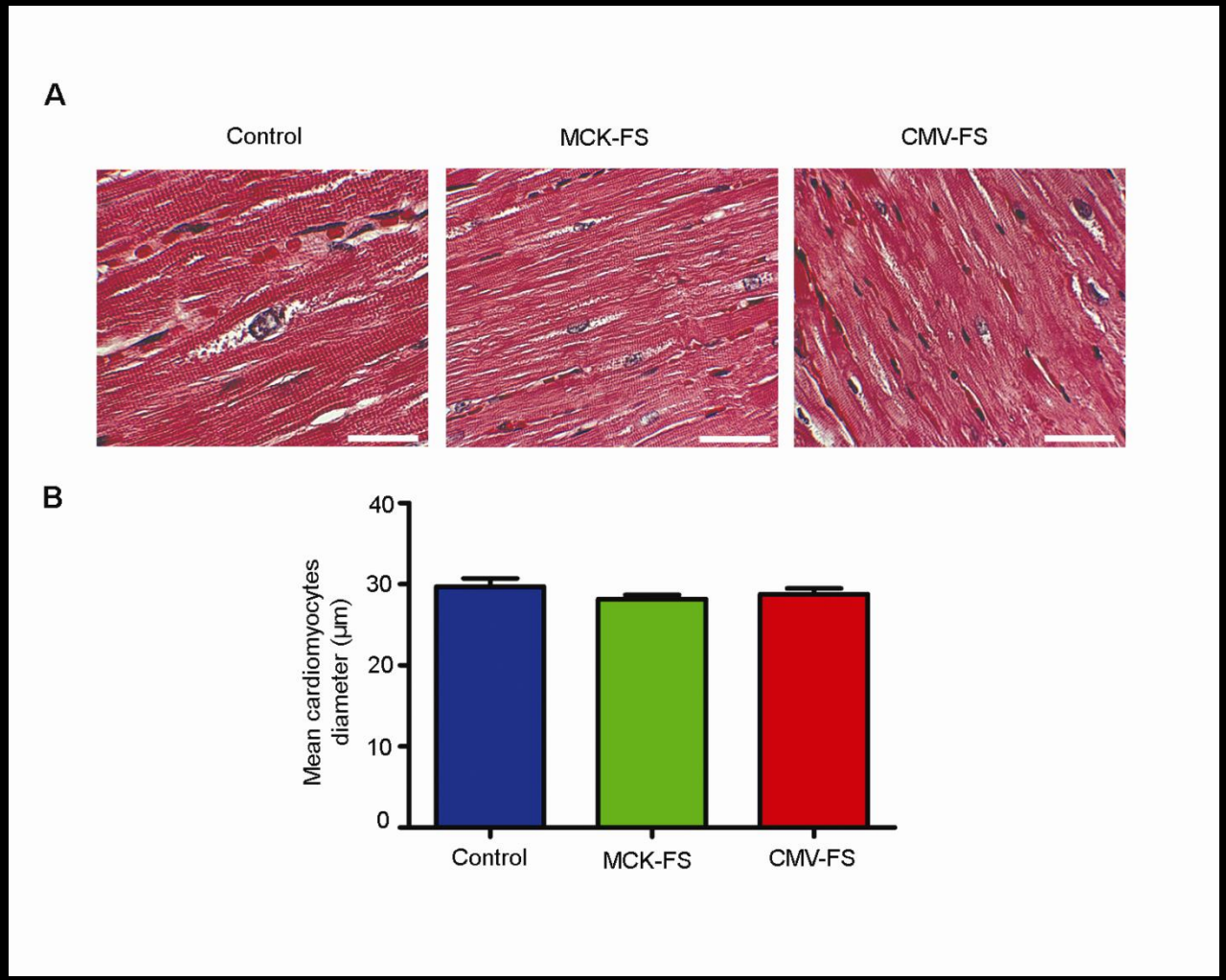
# Hormone Levels Post AAV1-FS Treatment NHP used in Pre-clinical Studies

| Table 3. Hormonal data of rAAV1- FS344 treated non-human primates | | | | | | | |
|---|---|---|---|---|---|---|---|
| Time Point | Animal | FSH (ng/ml) | | LH (ng/ml) | | Estradiol (ng/ml) | Testosterone (ng/ml) |
| Baseline | No | Males | Females | Males | Females | Females | Males |
| | 1 | 0.53 | 1.7 | 0.34 | 0.57 | 49.08 | 0.13 |
| | 2 | 0.74 | 1.39 | 2.21 | 1.03 | 17.65 | 8.28 |
| | 3 | 0.39 | | 0.35 | | | 1 |
| | 4 | 0.34 | | 0.18 | | | 0.12 |
| | | | | | | | |
| 5 months | 1 | 0.52 | 1.65 | 0.68 | 0.51 | 74.22 | 0.27 |
| | 2 | 0.42 | 1.39 | 0.78 | 2.35 | 61.3 | 9.02 |
| | 3 | 0.5 | | 0.55 | | | 4.99 |
| | 4 | 0.36 | | 0.25 | | | 1.67 |
| | | | | | | | |
| 15 months | 1 | | 1.21 | | 0.64 | 72.01 | |
| | 2 | 0.29 | 2.42 | 0.23 | 2.34 | 56.54 | 5.48 |
| | 3 | | | | | | |
| | 4 | 0.45 | | 0.42 | | | 7.42 |

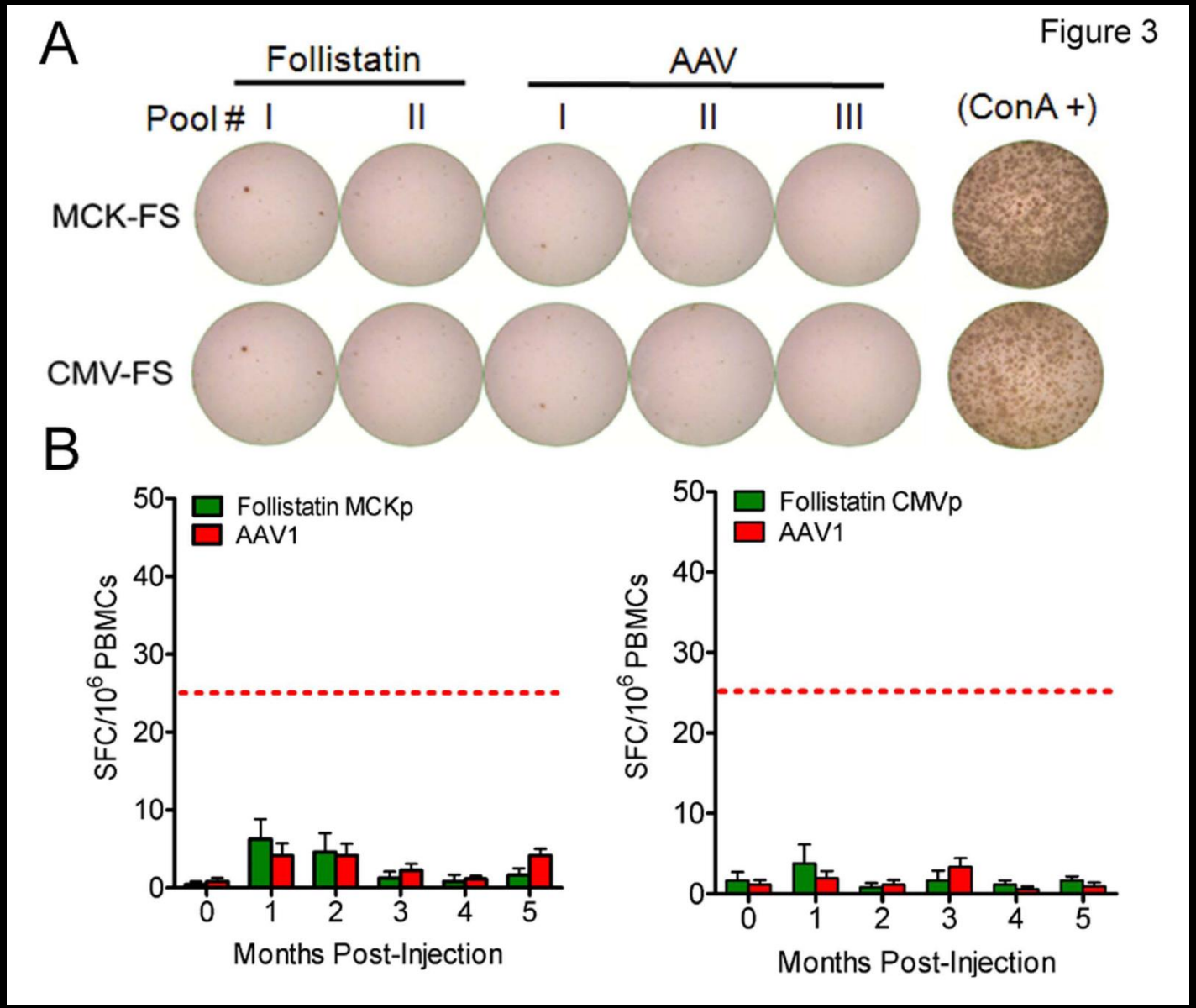
# Clinical Chemistries
# Monkeys used in Pre-clinical Studies

| Table 2. CBC Chemistry of rAAV1-FS344 treated non- human primates | | | | | | |
|---|---|---|---|---|---|---|
| | MCK-FS | | | CMV-FS | | |
| Parameter | Baseline | 5 months Post Injection | 15 months Post Injection | Baseline | 5 months Post Injection | 15 months Post Injection |
| Hgb (mg/dL) | 11.7 ± 1.2 | 12.3 ± 0.7 | 13.5 ± 0.6 | 12.9 ± 0.9 | 12.9 ± 0.3 | 12.6 ± 0.8 |
| WBC (K/cu mm) | 9.4 ± 3.6 | 11.0 ± 1.8 | 7.5 ± 1.7 | 13.2 ± 1.7 | 10.8 ± 2.8 | 15.5 ± 8.9 |
| Platelets (K/cu mm) | 444.7 ± 78.6 | 473.7 ± 101.5 | 448.5 ± 34.6 | 475.3 ± 21.2 | 470.0 ± 10.8 | 432.0 ± 39.6 |
| CK (U/L) | 282.3 ± 123.3 | 103.3 ± 34.0 | 261.0 ± 97.6 | 315.1 ± 436.8 | – | 141.0 ± 5.7 |
| ALT (U/L) | 29.7 ± 12.9 | 19.7 ± 2.1 | 31.5 ± 2.1 | 28.7 ± 10.3 | 21.7 ± 4.6 | 29.5 ± 6.4 |
| AST (U/L) | 35.3 ± 3.51 | 34.7 ± 9.9 | 37.5 ± 6.4 | 44.3 ± 11.4 | 31.7 ± 6.0 | 35.5 ± 4.9 |
| BUN (mg/dL) | 19.0 ± 1.0 | 12.3 ± 1.5 | 16.0 ± 1.4 | 16.3 ± 4.9 | 16.0 ± 4.4 | 18.5 ± 7.8 |
| Creatinine (mg/dL) | 0.5 ± 0.1 | 0.8 ± 0.1 | 0.7 ± 0.1 | 0.9 ± 0.2 | 0.9 ± 0.1 | 0.9 ± 0.1 |
| GGT (U/L) | 72.0 ± 28.8 | 92.0 ± 38.7 | 77.5 ± 51.6 | 77.0 ± 20.7 | 71.3 ± 18.2 | 75.0 ± 9.8 |

# Follistatin Treatment had **no** effect on Cardiac Tissue



# IFN-γ ELISpot to FS344 and AAV1



# Necropsies of NHP used in Pre-Clinical Studies

- Full necropsy on all monkeys
  - slides on each organ evaluated by a board certified veterinary pathologist blinded to treatment group (control vs FS)
- No treatment-related abnormalities found in heart, liver, lung, spleen, kidney, testis, ovary and uterus (5 &15 months)

# Pre-IND Meeting July 25, 2008

- Presented our Pre-Clinical Proof of Principle Studies
- Established a plan for toxicology-biodistribution study
- (initiated by Oct 1, 2009)
  - Required only mouse study in C57/Bl10
  - FDA accepted data from pre-clinical studies in NHP avoiding need for duplication
- rAAV.follistatin344 production is now complete at Nationwide Children's Research Institute cGMP for toxicology study.

Initial Reports on Tox. Completed by May 31, 2010

Final reports audited Sept 2010.

Recombinant DNA advisory committee presentation meeting June 7 2010.

FDA Agreed on design of clinical protocol
- Bilateral IM injection of quadriceps muscle
- Assessment of efficacy (muscle bx and strength) at six months

Plan is to submit Final IND to FDA on June 31, 2010. Trial can start 1 month following approval.

# Clinical Gene Therapy Trial
# AAV1.CMV.FS344
# Projected to begin in August 1, 2010

Initial Study:

Patients with proven sIBM with weakness of knee extensor muscles

3 dose cohorts  (n=3 patients per cohort)

Evaluate for Safety and Efficacy

Patients will return for follow up visits on
Days 7, 14, 20, 60, 90, 120, 180

-Quantitative strength measures of quadriceps muscles
and functional tests (stair climbing, walking 30 feet, getting up from chair)

MRI of quad muscles on day 180 will be compared with pre-treatment
Muscle biopsy on both quadriceps on day 180.

# The Kaspar lab