Systemic AAV-Mediated γ-sarcoglycan Gene Therapy for Treatment of Muscle Deficits in LGMD2C Mice

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Disclosures

E.R. Pozsgai, E.L. Peterson, D.A. Griffin, and L.R. Rodino-Klapac are employees of Sarepta.

Many LGMDs Are Caused by Defects in the Sarcolemmal Proteins That Comprise the Dystrophin-associated Protein Complex

- **Sarcoglycans** prevent muscle damage during contraction[1]
  - Mutations in any of the following 4 subunits of the sarcoglycan complex lead to muscular dystrophy[1]
    - **β-sarcoglycan**
    - **α-sarcoglycan**
    - **γ-sarcoglycan**
    - **δ-sarcoglycan**
  - Sarcoglycan deficiency leads to dystrophin deficiency[1]
- **Dysferlin** and **anoctamin-5** support muscle membrane repair[2]
  - Failed muscle repair leads to chronic muscle degeneration

SCHEMATIC OF SARCOLEMMA*

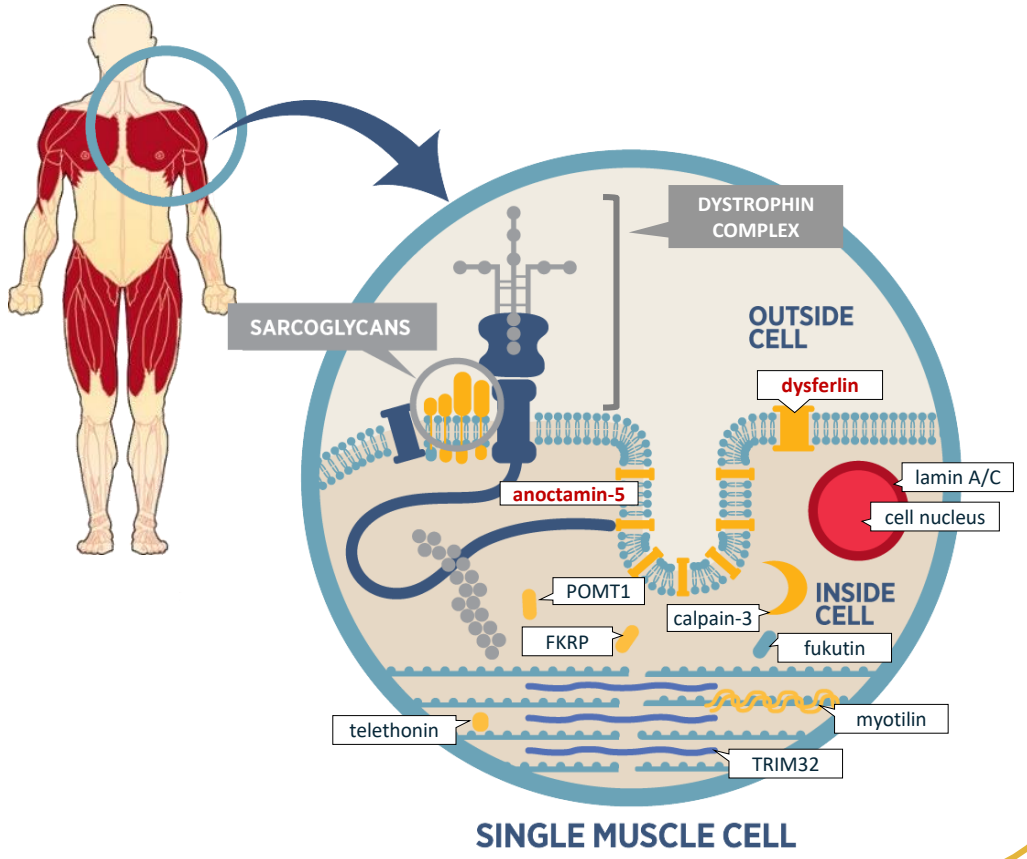
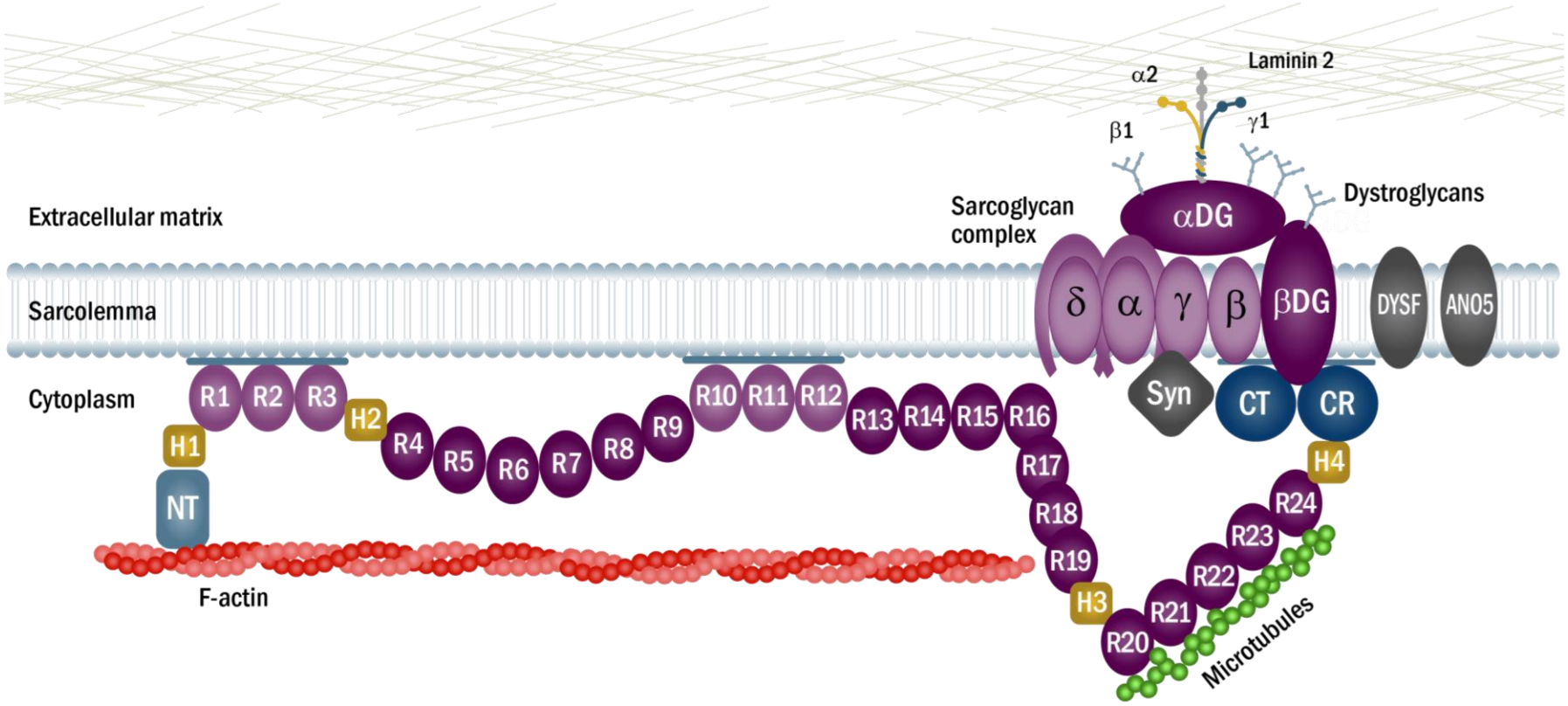


*Image adapted from the MDA website. Causes/Inheritance. www.mda.org/disease/limb-girdle-muscular-dystrophy/causes-inheritance. Accessed December 12, 2018.

1. McNally EM. The Sarcoglycans. Landes Bioscience 2000-2013. www.ncbi.nlm.nih.gov/books/NBK6317/. Accessed December 16, 2018. 2. Liewluck T, Milone M. *Muscle Nerve.* 2018;58(2):167-177.

The Dystrophin-Associated Protein Complex (DAPC)

- The relationship between dystrophin and the DAPC is both intricate and dependent
- The dystrophin complex **stabilizes the plasma membrane** of striated & cardiac muscle cells
- Loss of function **mutations** in the genes encoding dystrophin, or the associated proteins, **trigger instability of the plasma membrane and myofiber & cardiomyocyte loss**



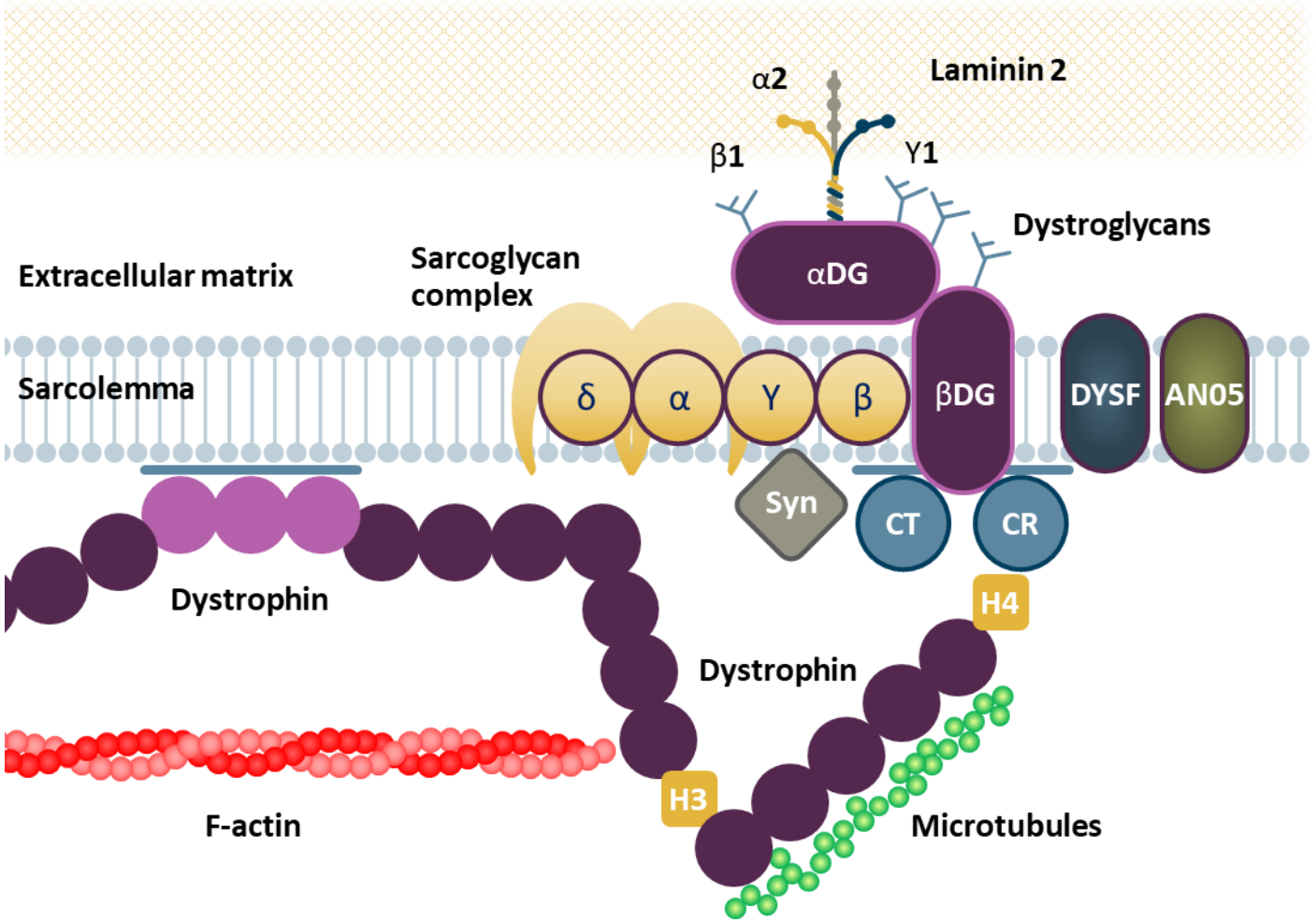
nNOS, neuronal nitric oxide synthase.

Image adapted from Fairclough RJ, et al. *Nat Rev Genet*. 2013;14(6):373-378.

The Sarcoglycan Complex: Essential Proteins of the DAPC

- **LGMD is caused by a broad range of mutations occurring in multiple genes that encode for proteins that play vital roles in muscle function, regulation and repair[1]**
- **Sarcoglycanopathies**
  - **Sarcoglycans prevent muscle damage during contraction[2]**
  - All 4 functional sarcoglycans must be present to form a functional sarcoglycan complex[3]

Gene/Protein[4]	Function[2]	Disease[4]
SGCA α-sarcoglycan	Stabilizes DAPC, prevents muscle damage during contraction	LGMD2D
SGCB β-sarcoglycan	Stabilizes DAPC, prevents muscle damage during contraction	LGMD2E
SGCG γ-sarcoglycan	Stabilizes DAPC, prevents muscle damage during contraction	LGMD2C
SGCD δ-sarcoglycan	Stabilizes DAPC, prevents muscle damage during contraction	LGMD2F



1. Mah JK, et al. *Can J Neurol Sci.* 2016;43(1):163-177. 2. McNally EM. The Sarcoglycans. Landes Bioscience 2000-2013. www.ncbi.nlm.nih.gov/books/NBK6317/. Accessed March 21, 2019. 3. Allen DG, et al. *Physiol Rev*. 2016;96(1):253-305. 4. Liewluck T, Milone M. *Muscle Nerve.* 2018;58(2):167-177.

LGMD2C (γ-Sarcoglycanopathy) Overview

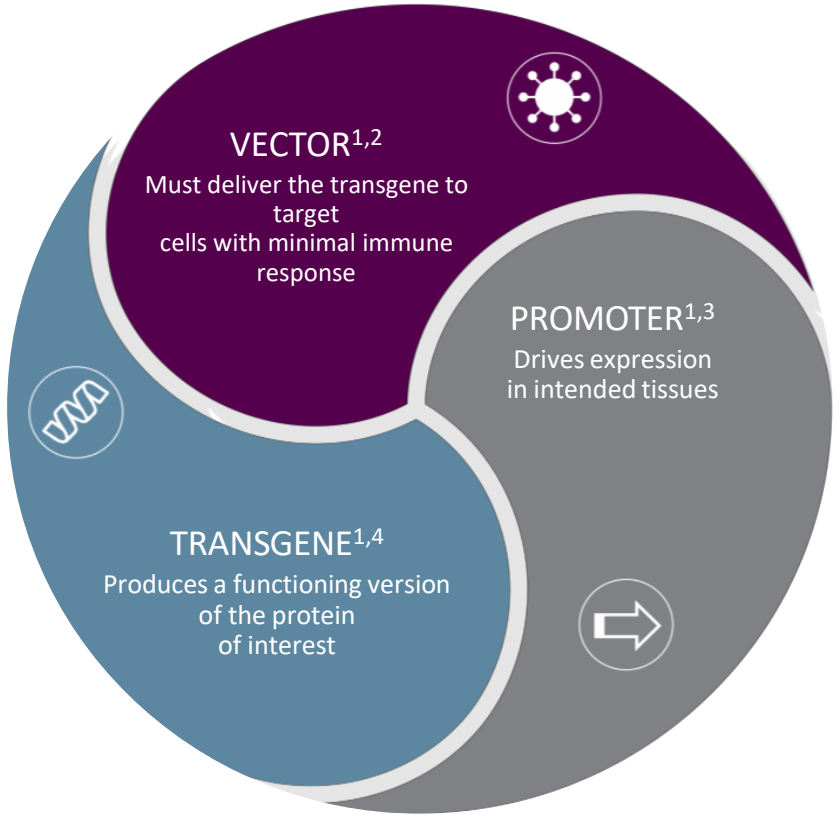
- Phenotype can present similar to that of DMD with severe, progressive proximal muscle weakness; however, some patients may exhibit a milder phenotype
- Symptoms may arise early in life, typically before age 10, and presentation may occur up to the second decade of life
- Common examination features include calf hypertrophy, scapular winging, macroglossia and lumbar hyperlordosis
- Diagnosis is typically established by a lack or reduction of γ-sarcoglycan expression on biopsies or genetic testing
- Respiratory failure and cardiomyopathy are common features and should be actively screened for
- Serum CK activity is generally moderate to high, but can range from 1000 U/L to 25,000 U/L

1. Liewluck T, Milone M. *Muscle Nerve*. 2018;58(2):167-177. 2. Wicklund. 3. Murphy AP, Straub V. Journal of Neuromuscular Diseases.2015;S8

Adeno-associated Virus (AAV)-Mediated Gene Transfer

- Adeno-associated viruses (AAV) are small, non-enveloped viruses that, unlike adenoviruses, have not been associated with human disease.[5,6]
- Several AAV serotypes have been identified, each with a different tissue tropism. This allows for specific tissue targeting with AAV-mediated gene therapies.[5]
- Delivers transgenes via nonintegrating, stable, extrachromosomal episomes to the nucleus, thereby limiting the risk of insertional mutagenesis seen with other viral vectors.[5]

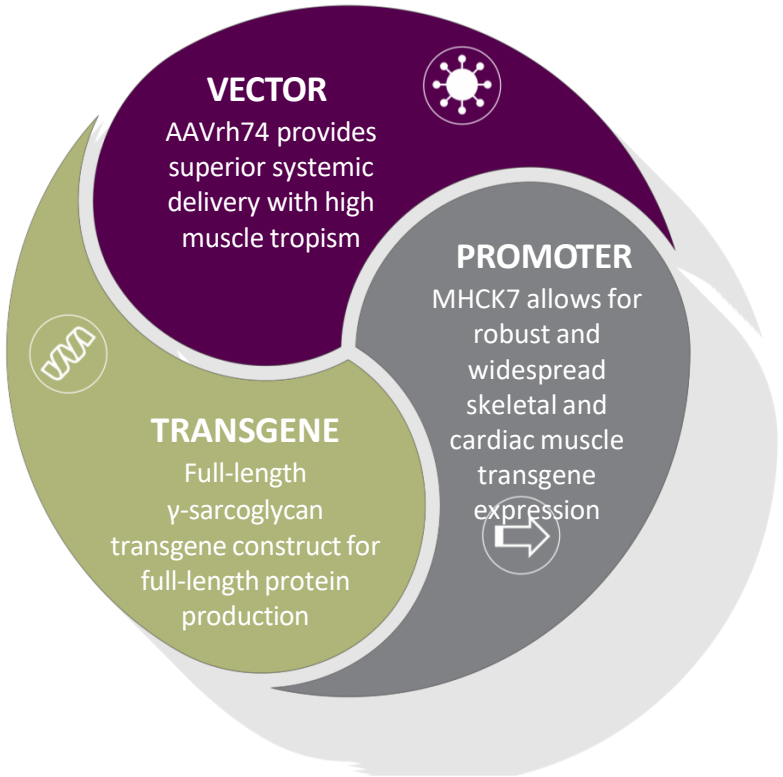
Essential Components of AAV Gene Therapy



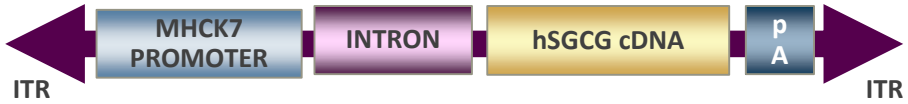
1. Naso MF, et al. BioDrugs. 2017;31(4):317-334. 2. US National Library of Medicine, Lister Hill National Center for Biomedical Communications. Genetics Home Reference. Help me Understand Genetics: *Gene Therapy*. Bethesda, Maryland: 2013. https://ghr.nlm.nih.gov/primer/therapy/genetherapy. Accessed August 29, 2018. 3. Zheng C, Baum J. *Methods Mol Biol*. 2008;434:205-219. 4. Chamberlain K, et al. *Hum Gene Ther Methods*. 2016;27(1):1-12. 5. Balakrishnan B, Jayandharan GR, *Curr Gene Ther*. 2014; 14:1-15. 6. Atchison RW et al. Science 1965; 149(3685): 754-6.

scAAVrh74.MHCK7.hSGCG for the Treatment of LGMD2C (γ-sarcoglycanopathy)

AAVrh74.MHCK7.hSGCC



scAAVrh.74.MHCK7.hSGCG

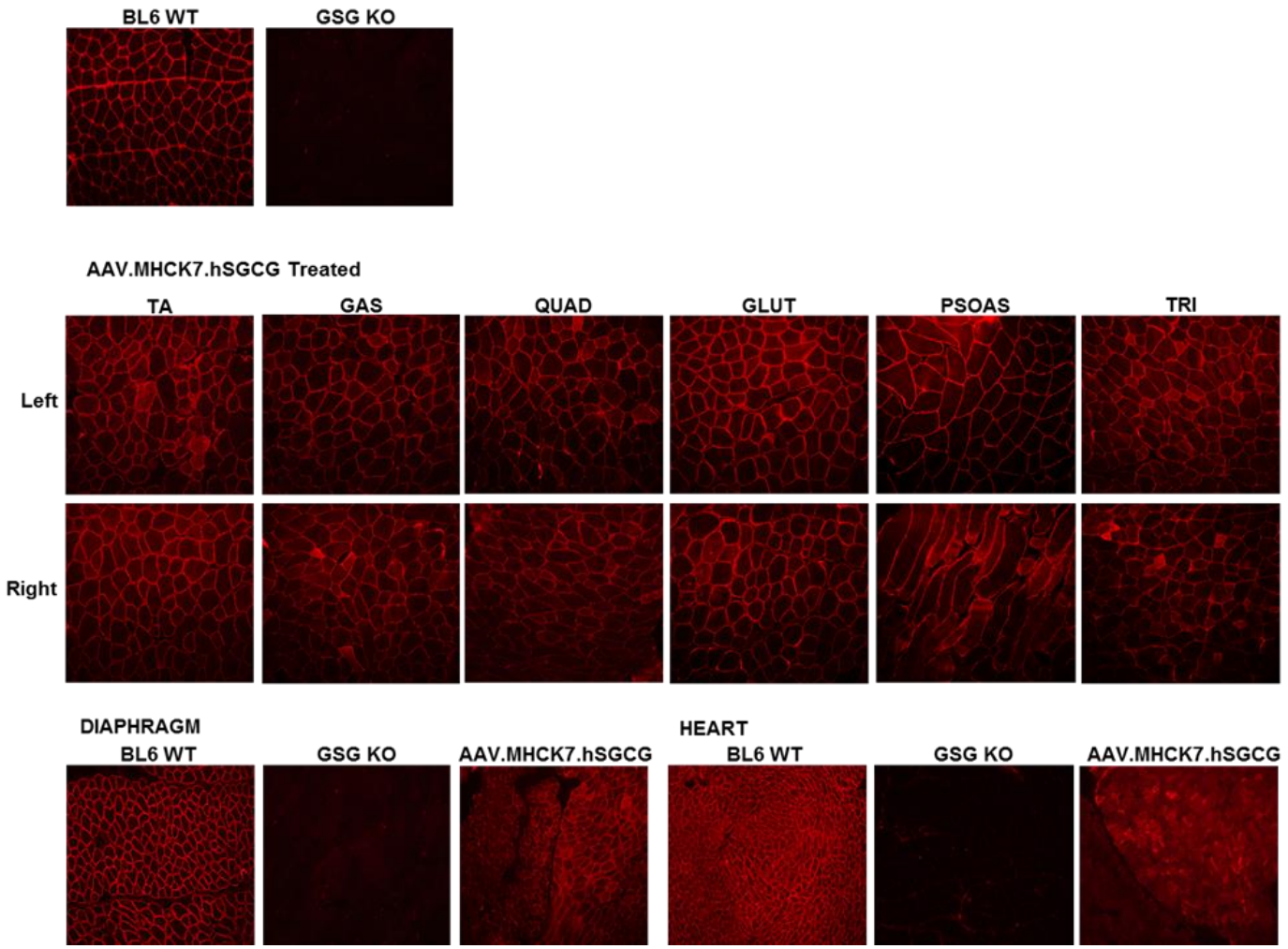


- **The scAAVrh74.MHCK7.hSGCG construct was designed to restore functional full-length γ-sarcoglycan to muscle cells**
  - **AAVrh74** was chosen based on skeletal muscle transduction in preclinical testing[1-3]
    - Because muscle cells do not divide, AAVrh74-transduced muscle fibers may theoretically be protected indefinitely
    - AAVrh74 provides systemic delivery, including to cardiac muscle
    - Preclinical data support single-administration hypothesis
  - The **MHCK7** promoter is optimized for the desired expression in skeletal and cardiac muscle expression regulated by α-MHC to enhance cardiac expression[2,3]
  - **γ-sarcoglycan transgene is a Full-Length** gene construct for γ-sarcoglycan[4]

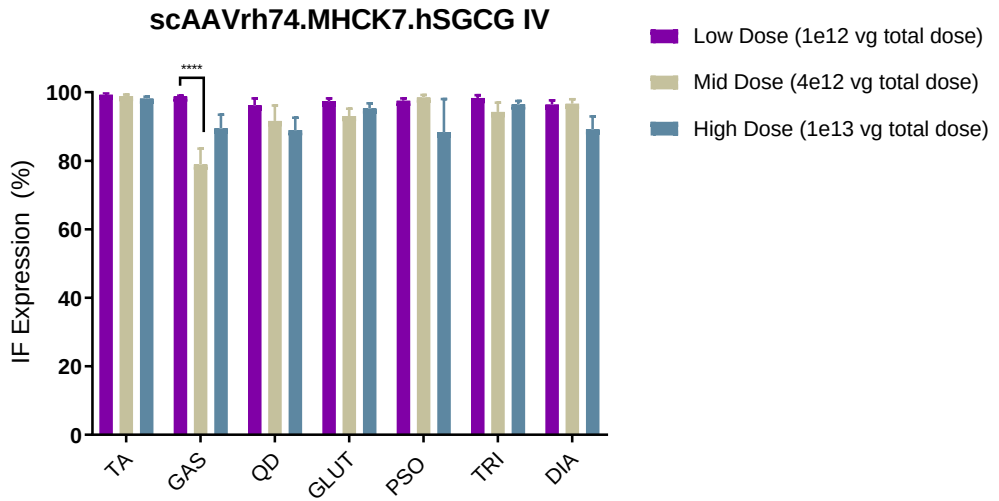
ITR, inverted terminal repeat; hSGCB, human β-sarcoglycan; pA, poly-A tail

1. Chicoine LG et al. *Mol Ther* 2013;22:338-47. 2. Pozsgai ER. *Mol Ther* 2017;25:855-69. 3. Salva MZ, et a. *Mol Ther.* 2007;15(2):320-329. l 4. Colella P, et al. *Mol Ther Methods Clin Dev* 2017;8:87-104.

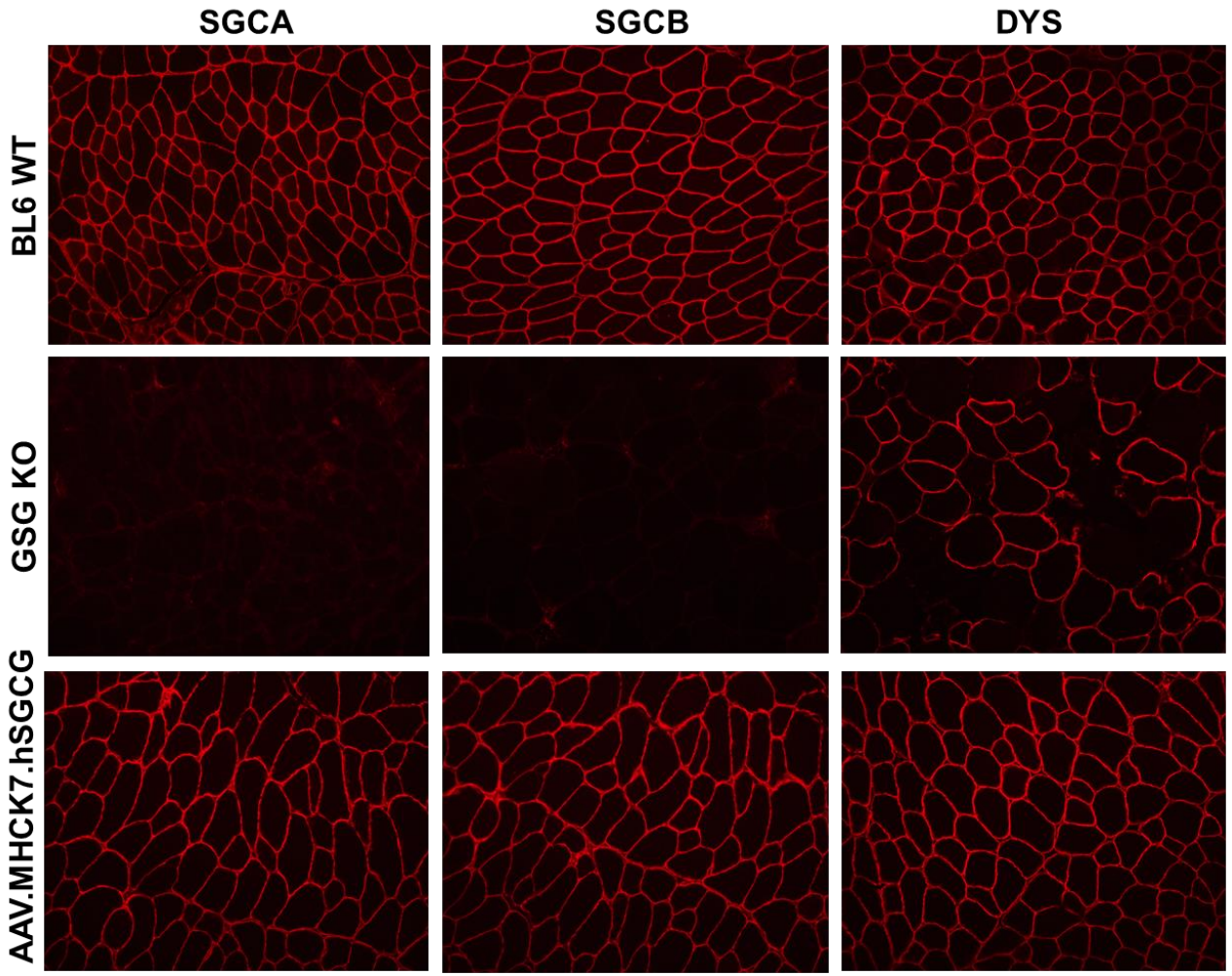
Systemic scAAVrh.74.MHCK7.hSGCG Restores γ-Sarcoglycan Expression in *GSG KO* Mice



- **hSGCG Expression in Three Dosing Cohorts 12 Weeks Post IV Gene Delivery**
- **Widespread Expression in Skeletal and Cardiac Muscle**
- **hSGCG Correctly Localizes to Sarcolemma**



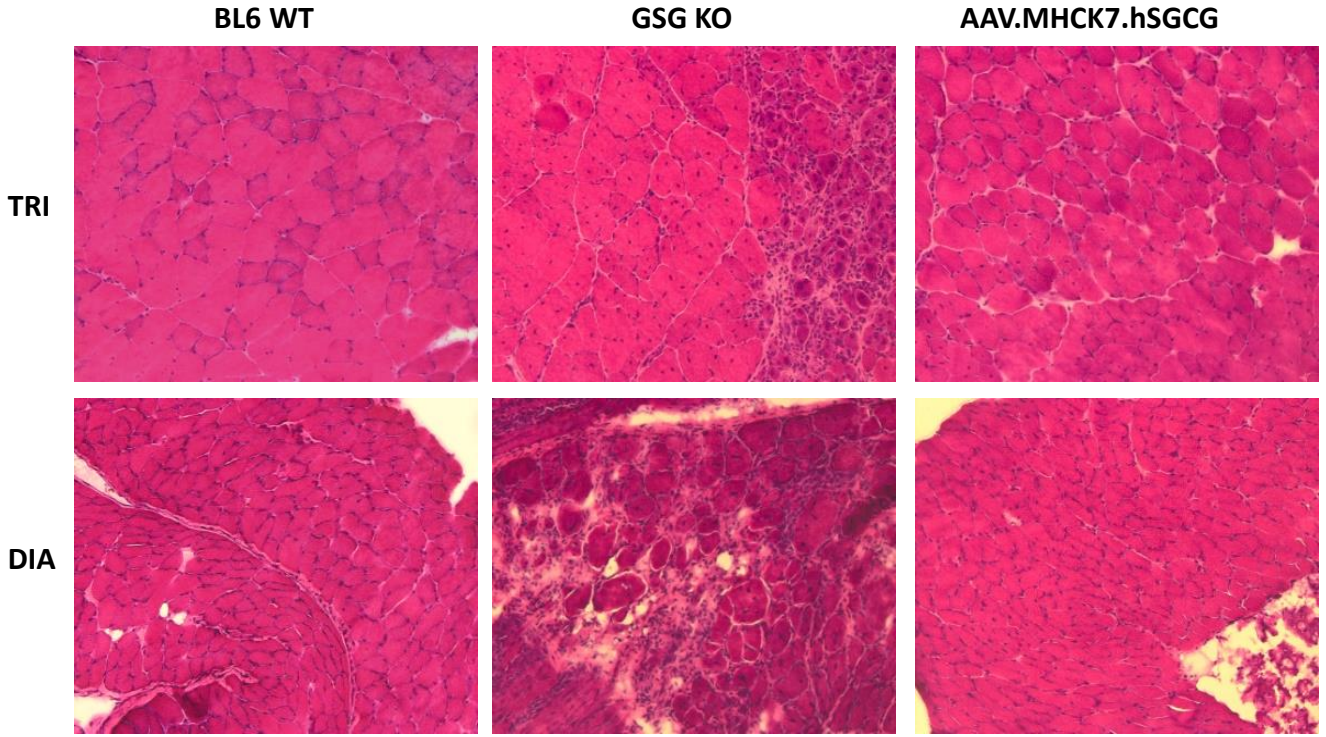
Restoring γ-Sarcoglycan At the Membrane Leads to Restoration of Other DAPC Components

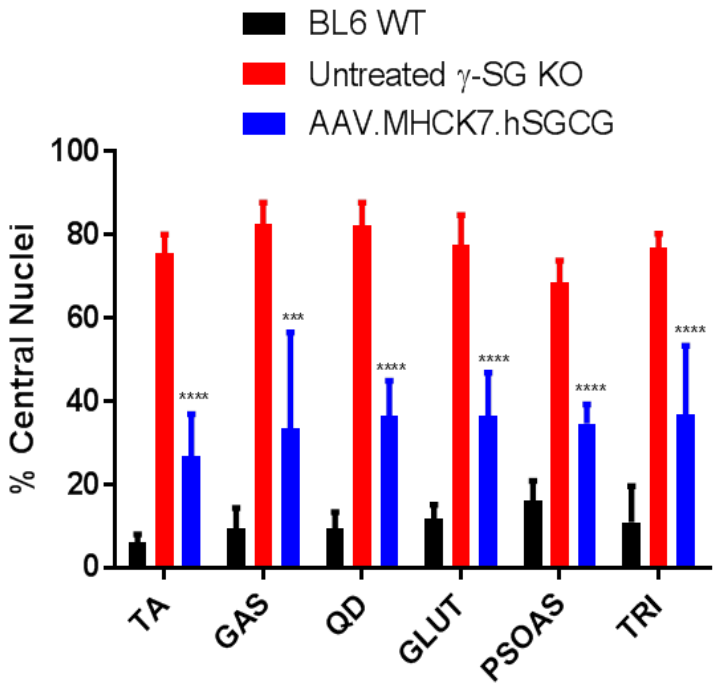


- **Re-assembly of dystrophin associated protein complex (DAPC) at sarcolemma**
  - GSG KO mice show absent or reduced sarcolemma expression of α-sarcoglycan, β-sarcoglycan, and dystrophin.
  - Treatment with AAV.MHC7.hSGCG
    - Increased α-sarcoglycan and β-sarcoglycan subunit expression at the sarcolemma in GSG KO mice
    - Increased expression of dystrophin at the sarcolemma in GSG KO mice

Improved Muscle Morphology Following γ-Sarcoglycan Gene Transfer

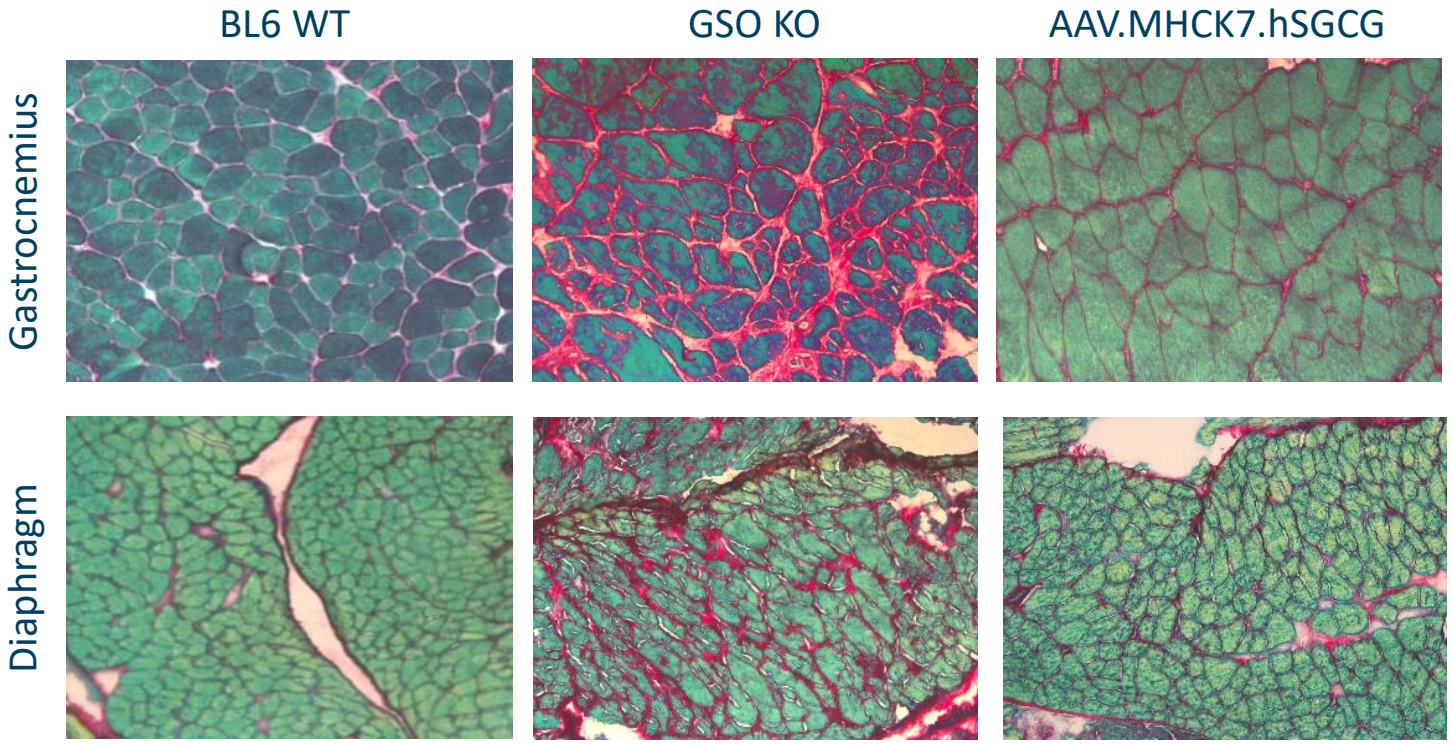
55% average reduction in central nucleation across skeletal muscle tissue of GSG KO mice after γ-sarcoglycan gene transfer



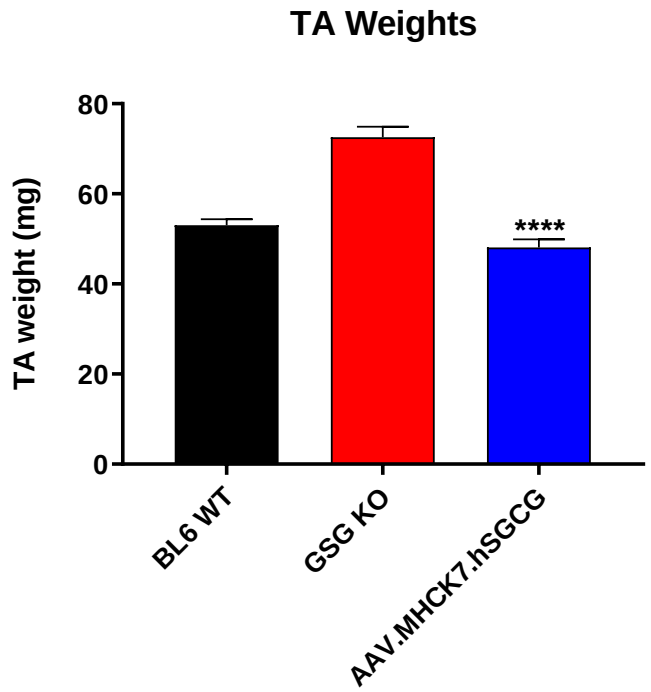


Systemic γ-Sarcoglycan Gene Delivery to Diseased GSG KO Mice Reduced Muscle Fibrosis

Reduction in Fibrotic Tissue Deposition and Normalized Muscle Weight in Treated Muscle

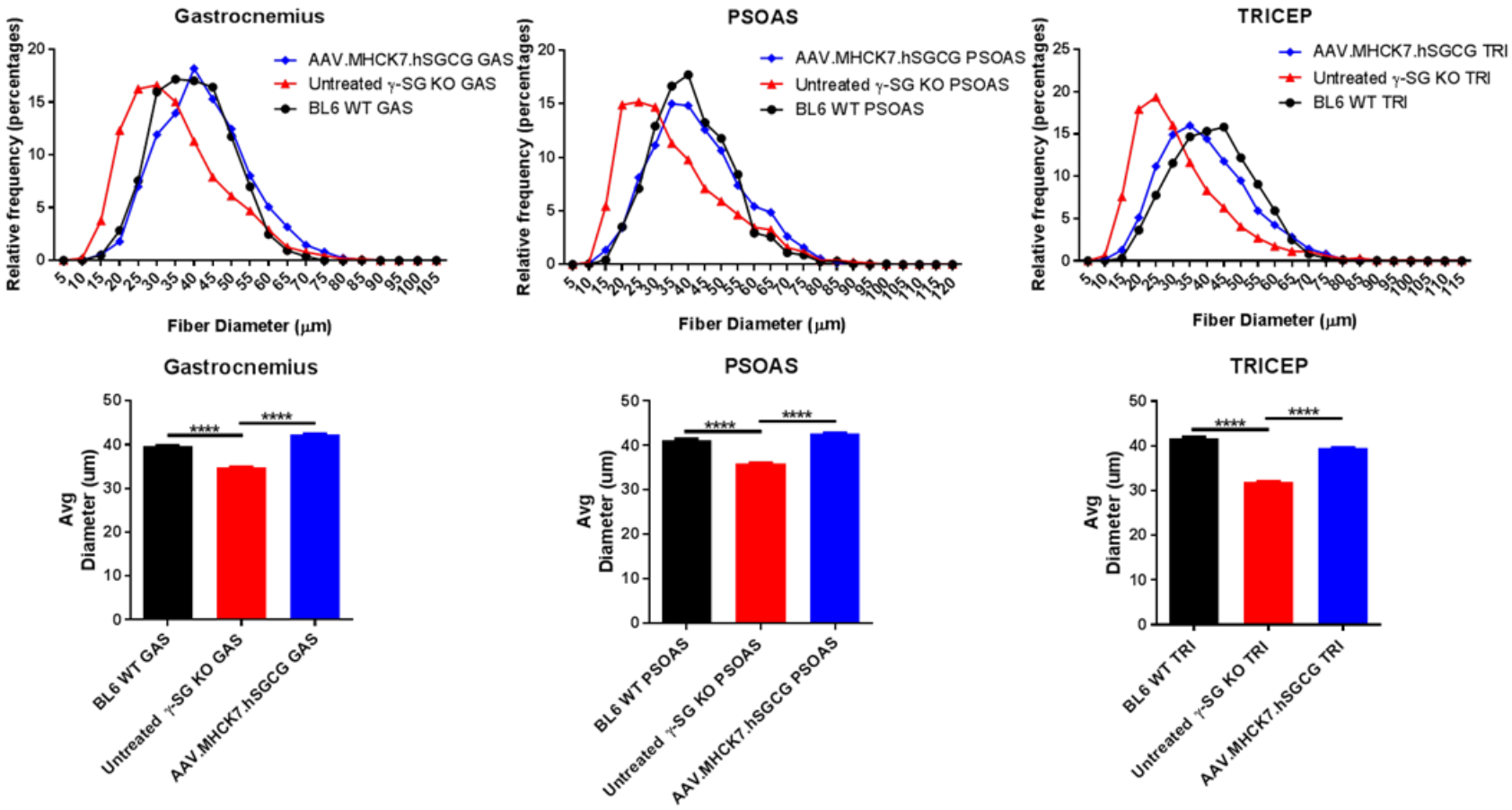


Green: Muscle
Pink: Fibrosis/Scar Tissue

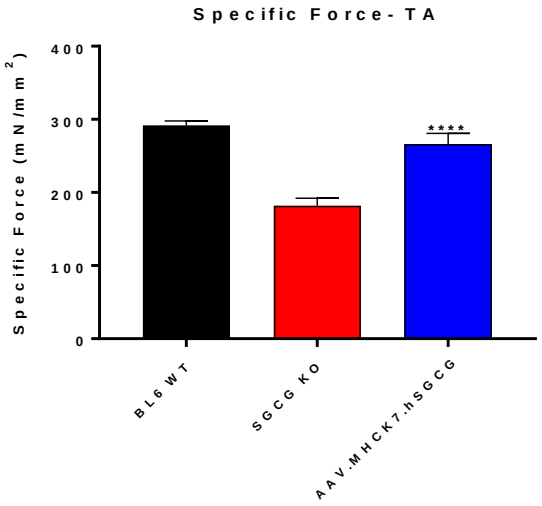


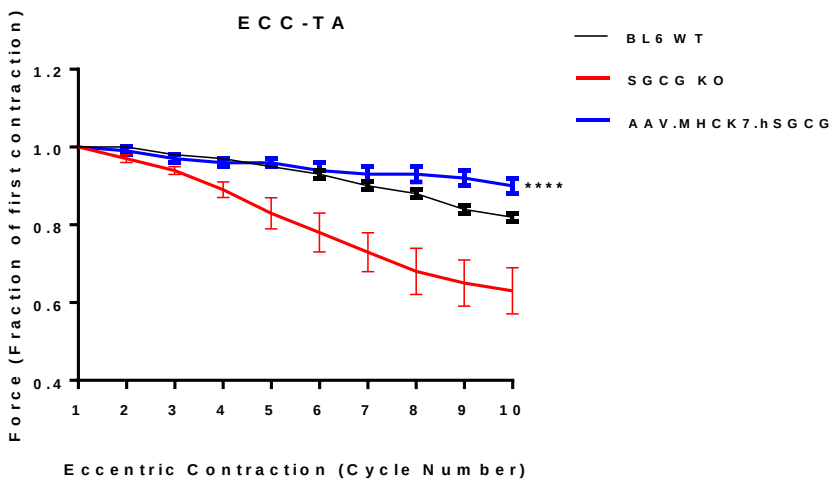
Expression of γ-Sarcoglycan Transgene in GSG KO Muscle Normalizes Muscle Fiber Distribution and Increases Muscle Fiber Size

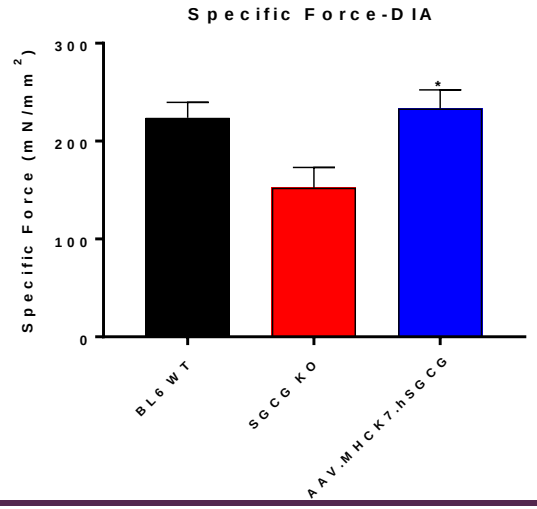
Distribution of Fiber Size is Normalized After hSGCG Gene Delivery

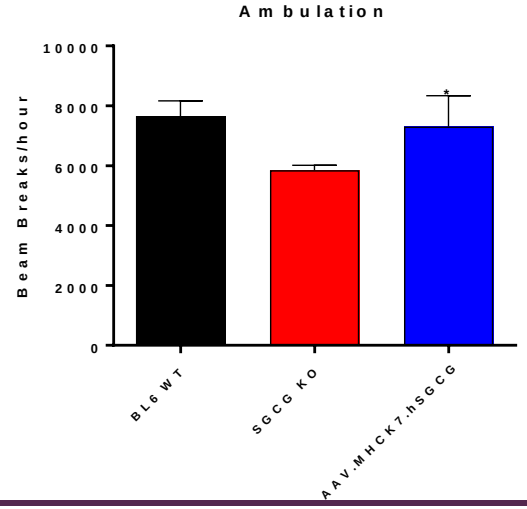


Functional Improvement Resulting from Systemic γ-Sarcoglycan Gene Transfer





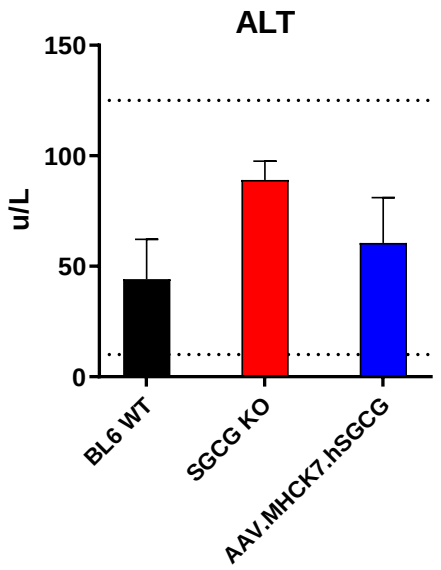


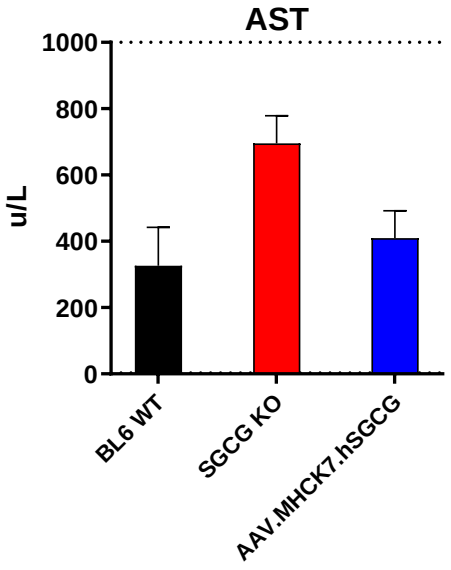


- **Functional benefits to skeletal muscle**
  - GSG KO mice show impairments in muscle force production, lack of resistance to contraction-induced injury and reduction in ambulatory activity.
  - Treatment with AAV.MHC7.hSGCG
    - Improved specific force in the TA and DIA
    - Improved resistance to contraction-induced injury
    - Restored ambulatory activity to normal wild type levels

No Observed Adverse Effects in Pre-clinical Safety Studies

Serum Liver Enzymes Reduced and No Histopathological Findings Following Gene Transfer





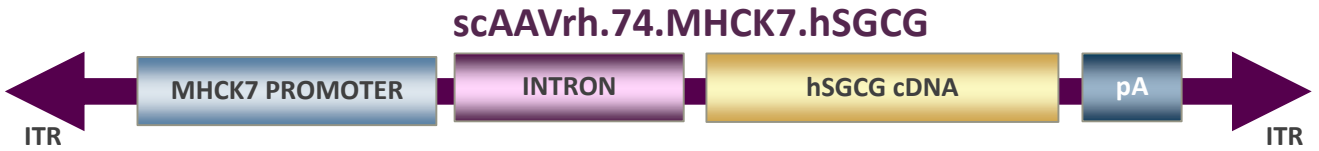
Test Article (vector dose)	Age at Injection	Treatment Length	Tissues Analyzed	Histopathology
scAAVrh.74.MHCK7.hSGCG (1e13vg total dose)	4 weeks	12 weeks	Skeletal Muscles, Heart, Lungs, Kidney, Liver, Spleen, Gonads	*NF

*NF – No histopathological findings in independent veterinary review

Conclusions from γ-Sarcoglycan Pre-Clinical Studies in GSG KO Mice

- GSG KO mice are complete KO of SGCG, recapitulate LGMD2C disease phenotype
- Widespread high level expression of hSGCG transgene
- Upregulation and re-assembly of DAPC
- Improved muscle morphology following γ-sarcoglycan gene transfer
- Restored muscle strength and increased ambulation in treated GSG KO mice
- No Observed Adverse Effect Level (NOAEL) in treated GSG KO mice

AAV γ-Sarcoglycan Gene Therapy Program Synopsis



Design Consideration	Driven By	Output
Tissue targeting (skeletal and cardiac)	AAVrh74 Promoter	Transduction and expression in muscles and heart
No Safety Events	AAVrh74 Full-Length Transgene	No Toxic Histopathology
Correct localization (hSGCG and DAPC proteins)	Promoter Transgene	Restoration of DAPC
Function	Transgene	Improvements in specific force, resistance to injury, ambulation

Thank you